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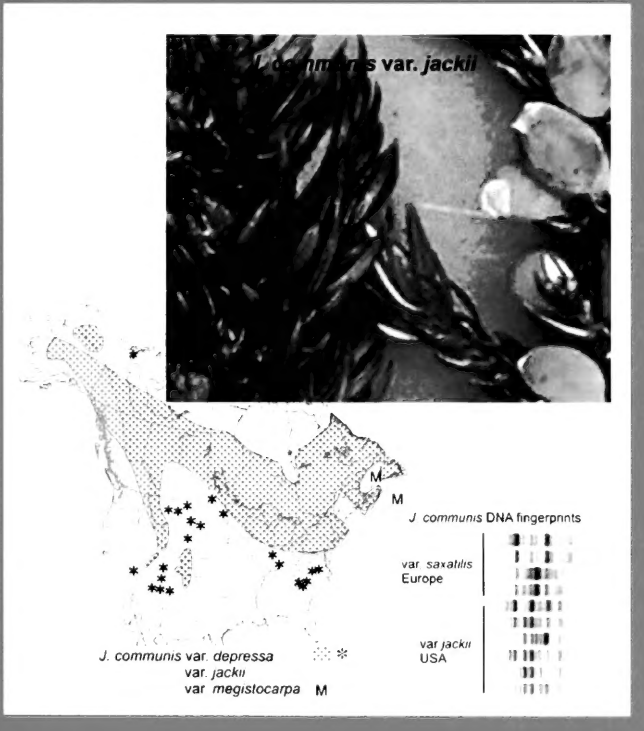

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Robert P. Adams
Phytologia, Box 727, Gruver, TX 79040
Robert_Adams@baylor.edu

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Cover Photo - composite photo featuring *Juniperus communis* var. *jackii* from Adams and Nguyen, pp. 43 - 57.

Authors are encouraged to submit a color photo for use on the cover.

TWO MORE ALIEN GRASSES NOW AT HOME IN THE CONTINENTAL U. S. A.

John R. Reeder and Charlotte G. Reeder
Herbarium (ARIZ), Herring Hall, P.O. Box 210036,
University of Arizona, Tucson, AZ 85721

ABSTRACT

Two alien grasses, formerly unknown in this country, are reported to be growing spontaneously in the continental U.S.A. *Pogonarthria squarrosa*, a native of Africa, is growing naturally in the foothills of the Huachuca Mts., Cochise County, Arizona, and *Sporobolus creber*, native to Australia, is established on a ranch in Glenn County, California.

KEY WORDS: Grasses, alien species, *Pogonarthria*, Africa, *Sporobolus*, Australia.

During the early part of the 20th century, numerous foreign grasses were introduced into the southwestern US with the object of range improvement and/or erosion control. Many of these were from Africa, and some have now become well established. Familiar examples are *Eragrostis curvula* (Schrad.) Nees, both var. *curvula* and var. *conferta* Nees; *E. echinochloidea* Stapf; *E. lehmanniana* Nees; and *E. superba* Peyr. In fact, *E. lehmanniana* is so well adapted that it is now one of the most common grasses in southern Arizona.

Authors' note: This note was originally submitted in 2000, in advance of the publication of the genera *Pogonarthria* and *Sporobolus* in vol. 25 of Flora of North America (Barkworth et al. 2003), however the manuscript was lost during the transition to new editorship of *Phytologia*. While the FNA volume has long since been issued, the historical detail regarding the advent of these species remains relevant and is complementary to those treatments.

One African grass that was grown in the experimental grass gardens, but was not known to have become naturalized, is *Pogonarthria squarrosa* (Licht.) Pilger. In the ARIZ herbarium are three specimens [as *P. falcata* (Hack.) Rendle]. The oldest was collected by [J.J.] Thornber and has the following information on the label "Seeds from South Africa. Exp. Sta. Grass Garden, Tucson. 11/12/[19]06." There are two other sheets, one with the following label data: "E.W. Hardies A-3290, City Farm, Tucson, Ariz. Sep 27, 1937." It has a stamp: "Det. at U.S. Nat. Herb." The third sheet bears only the name of the plant, the collector's name (A. R. Purchase) and the date: 8/15/38. There is little doubt that the Hardies and Purchase specimens were grown from the same seed source, perhaps are part of a single clump. The oldest specimen, made some 30 years earlier, may have been grown from a different seed source.

We have found no record to indicate that any attempt was made to grow the species outside the garden, and ARIZ has no specimens other than those indicated above. Checking with colleagues at UNMR and TAES, we were informed that those herbaria have specimens of this species collected in Africa or grown in the garden, but none from plants which were growing naturally in New Mexico or Texas.

In early 1999, Patty Guertin brought us a few inflorescences of a grass which Barbara Alberti at the Coronado National Memorial had given her for help with the identification. When Patty, a volunteer worker at the Memorial, was unable to supply a name for it, she came to us for assistance. We soon realized that it was *Pogonarthria squarrosa*, a member of the Cynodonteae, and a native of eastern and southern Africa. At the time neither Ms. Alberti nor Ms. Guertin knew who had brought in the specimen, nor its source. We were very interested, because if it were growing spontaneously in Arizona, it would be a new record for the State and probably for the nation. It was not until mid-March 1999 that the information was forthcoming. It turned out that a Dr. Jay Davenport, who resides in a new housing development just south of Sierra Vista, had found the grass growing near his property and was curious as to its identity. Finally, he returned to the Memorial to inquire whether anyone had been able to name his grass.

We were supplied with the name and address of Dr. Davenport and were able to visit him at his home. He had seen only a few clumps of the grass, and had no idea as to how much might be present. He told us he was intrigued by the inflorescence, which reminded him of the double helix of the DNA molecule. He had several inflorescences in a vase by his fireplace. Even though at that time the grasses were dry and brown, and had shed their spikelets, we were able to identify a few clumps in the area, and concluded that there might be a sizable population of the *Pogonarthria* among the other grasses.

The area which includes the Davenport property is rather extensive, and apparently was formerly a ranch. The vegetation is largely grassland, which includes – along with the natives – a fair proportion of *Eragrostis lehmanniana.* For the present, at least, the houses are scattered, and much of the grassland remains. We made several visits through the summer, especially during the "monsoon season" in order to learn more about the *Pogonarthria*, and to collect herbarium specimens. It appears that it was just fortuitous that Dr. Davenport bought the particular parcel of land that he did, and that he was curious concerning this striking grass. As it turned out, his property is at the edge of the area in which most of the *Pogonarthria* is present.

We have been unable to learn when this alien grass was introduced into this area, or whether its introduction was purposeful or accidental. It is now clearly well established, and seems to be competing well with the other grasses. The area in which we observed it covers some 5 acres or more. It tends to occur in colonies of from 3 to 10 meters in extent. When it has bloomed and is ripening it is easily seen among the other grass because of its reddish-golden color. How long this species may persist here, especially under the threat of real estate development, is a matter of conjecture. For the present, at least, it is well established, and must be considered a part of the Arizona grass flora. It appears to be a new record for the US as well.

There is some indication that the *Pogonarthria* may be extending its area. We observed some scattered clumps along the roadside near the Davenport site, and this past summer Erika Geiger found several

plants along the road just west of the Air Force Aerostat site on Fort Huachuca, some distance north of the Davenport area.
Specimens Cited: USA. ARIZONA. Cochise County: Huachuca Mts. Foothills, S of Sierra Vista. Grassy area (probably former ranchland) now being developed as residential property. Small colony on and adjacent to home of J. Davenport at 1477 Loma Lane, elev. 1450 m. Well established and competing well with other grasses. 17 Aug 1999. J.R. & C.G. Reeder 9739 (ARIZ) (Fig. 1). Same general area but at this time it is evident that there are many more colonies than formerly suspected, some several meters in diameter. 2 Sep 1999. J.R. & C.G. Reeder 9768 (ARIZ, NMCR, RSA, TAES, TEX, UC, UTC). Fort Huachuca Military Reservation, along road W of Air Force Aerostat site (E border of FHMR). Small population (+ 100 individuals). 18 Aug 1999. Erika Geiger s.n. (ARIZ).

Another interesting grass which seems to be new to the USA is *Sporobolus creber* De Nardi. In early 1999, a single specimen was sent to us by A.C. Sanders (UCR) along with other grasses for our determinations or verification. This grass is native to Australia, and is a member of the *S. indicus* complex, closely related to *S. elongatus* R.Br. Although Simon and Jacobs (1999) recognize both species as distinct, Baaijens and Veldkamp (1991) treated them as varieties of *S. indicus* (L.) R.Br. In most modern treatments of *Sporobolus* in American floras, *S. creber* would key to *S. indicus*. However, *S. creber* has the following differences: the inflorescence branches are shorter than the internodes (often conspicuously so) particularly in the lower part; the anthers are 2, rather than 3; and the spikelets as well as the grains are somewhat smaller.

Although our specimen (cited below) was collected in 1995, we have information that the species persists, and is spreading. It appears to be well established on the Holzapfel ranch, at least, and must be considered a member of the California grass flora. We had hoped to obtain specimens for distribution through a fellow botanist in California during this past year, but were unsuccessful.

The label of our specimen also includes the following remark: "Has been present in small amounts (ca. 50 plants in one pasture) for several years, suddenly increased greatly and spread to other pastures.

Cattle will not eat this grass." Simons and Jacobs (1999) do not discuss this grass vis-a-vis cattle grazing, but in a personal communication (e-mail, 4/2000) Jacobs informs us that in Australia the plant can be somewhat "weedy." The tough fibrous nature of the old leaves retained on the plants often cause cattle to lose teeth and therefore stock tend to avoid them. He adds that in Australia the species can be readily removed by cultivation and the addition of fertilizer.

Specimen Cited: USA. CALIFORNIA. Glenn County: Sacramento Valley, Holzapfel Ranch, 4 mi S of Willows and 5 mi E on County Rd 60, just N of Sacramento Valley Wildlife Refuge, perennial, elev. ca. 30.5 m. 20 Sep 1995. Roy Holzapfel 1 (ARIZ) (Fig. 2).

ACKNOWLEDGMENTS

The assistance of Bryan Simon and Surrey Jacobs for information on the Australian *Sporobolus creber* and related taxa is much appreciated. S.A. Renvoize (K) kindly provided us with a photo of an isotype of *S. creber* and copies of authentic specimens of *S. elongatus*. We also thank Mary Barkworth and Philip Jenkins who reviewed the manuscript. Kathryn Mauz provided the photographs, and was most helpful in formatting the manuscript for publication.

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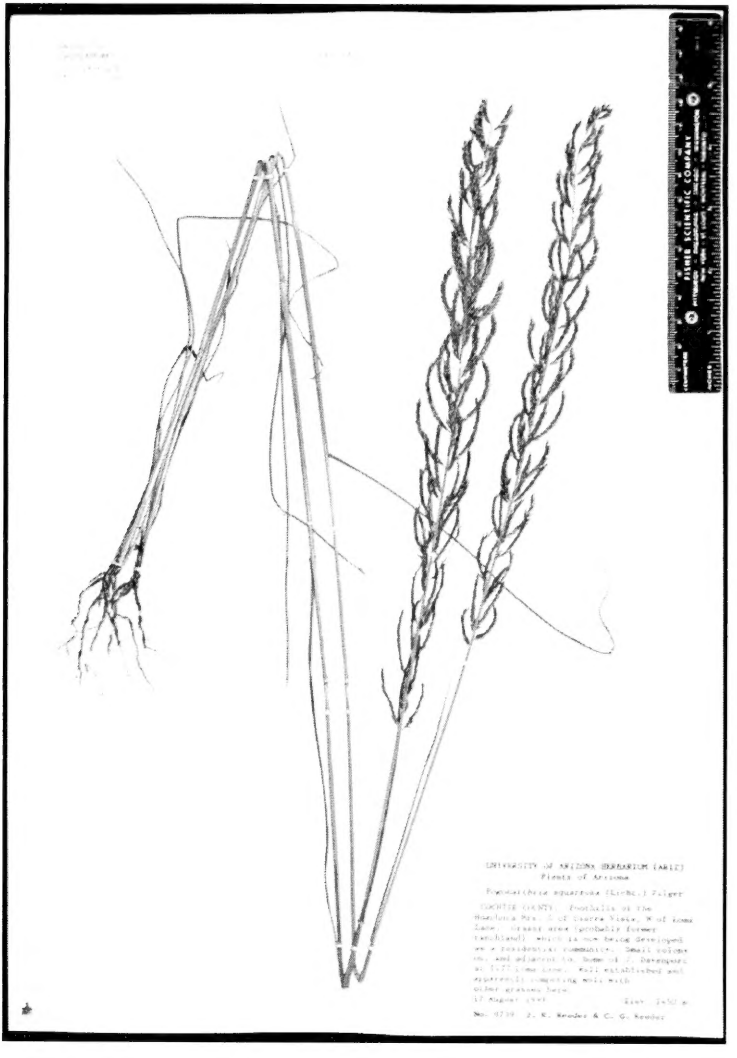

Fig. 1. *Pogonarthria squarrosa* (Licht.) Pilger. Near Sierra Vista, foothills of Huachuca Mts, Arizona, 17 Aug 1999 (ARIZ).

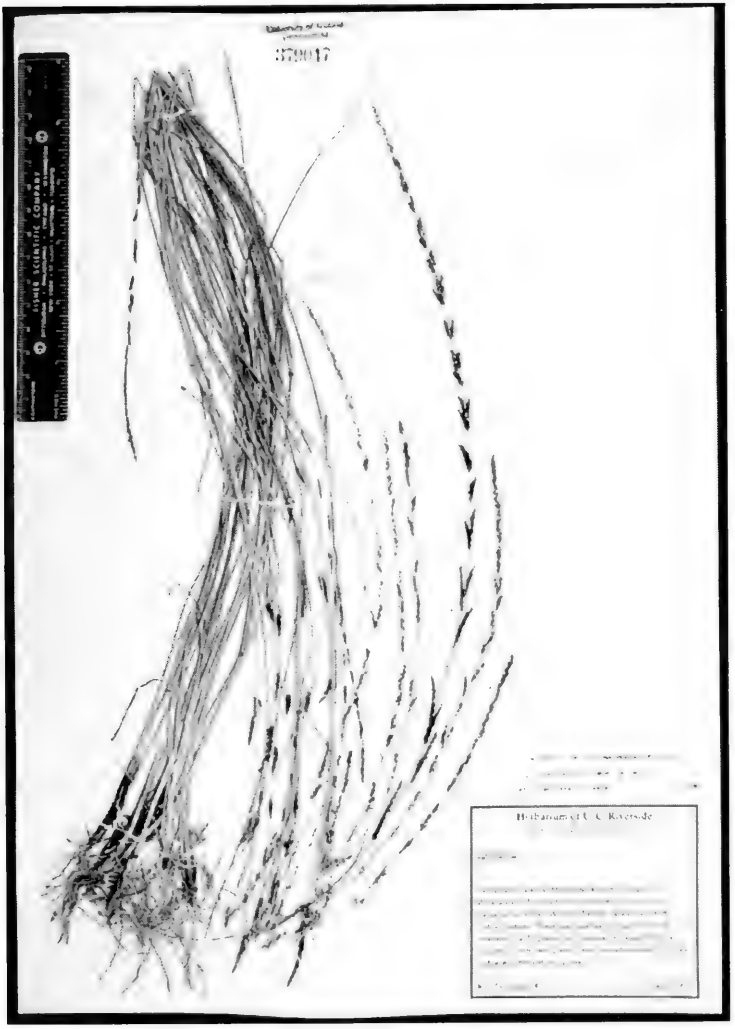

Fig. 2. *Sporobolus creber* De Nardi. Holzapfel Ranch, Sacramento Valley, California, 20 Sep 1995 (ARIZ).

PLEISTOCENE INFRASPECIFIC EVOLUTION IN *JUNIPERUS ASHEI* BUCH.

Robert P. Adams
Baylor University, Gruver Lab, P.O. Box 727, Gruver, TX 79040 USA
email Robert_Adams@baylor.edu

Lori E. Baker
Baylor University, Anthropology Forensic Science and Archaeology Department, P.O. Box 97388, Waco, TX 76798, USA

ABSTRACT

The Pleistocene and recent distributions of the taxa are discussed. A new variety of *Juniperus ashei*, *J. ashei* Buch. var. *ovata* R. P. Adams is recognized from west Texas and northern Mexico. It differs from typical *J. ashei* in having whip leaf glands that are oval to elongate rather than hemispherical, smaller female cones, smaller seeds and more seeds per cone. In addition, the leaf oil of var. *ovata* is higher in α-pinene, myrcene, limonene, γ-terpinene, bornyl acetate and elemol but lower in linalool, trans-sabinene hydrate, trans-p-menth-2-en-1-ol, camphor, trans carveol and carvone. RAPDs analysis revealed that the varieties are distinct in their DNA. It is hypothesized that var. *ovata* is a pre-Pleistocene relict, while var. *ashei* is a recently (late Pleistocene - Holocene) derived taxon.

KEY WORDS: *Juniperus, J. ashei* var. *ovata,* RAPDs, essential oils, Pleistocene distributions, Cupressaceae

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Juniperus ashei is a small tree that grows abundantly on limestone on the Edwards plateau in central Texas with disjunct populations on limestone in Arkansas, Missouri, and Oklahoma as well in Coahuila, Mexico (Fig. 1). The Edwards Plateau (limestone) region of central Texas supports dense populations covering millions of acres, whereas the disjunct populations (Fig. 1) often have almost pure stands of *J. ashei*, that may cover only a few acres.

Studies of geographic variation in *Juniperus ashei* have shown that the species has divergent populations in the semi-arid margins of its

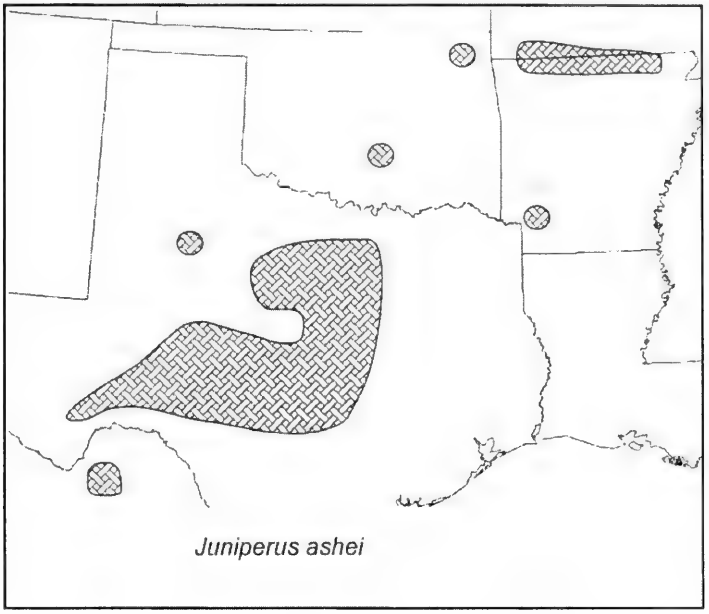

Figure 1. Distribution of *J. ashei*, adapted from Adams (2004). Note the disjunct populations.

range (Adams, 1977, 2004). Both leaf terpenoids and morphology were subjected to canonical variate analyses (Adams, 1977). These coordinate scores, when plotted onto maps, show a sharp divergence in Mexico and west Texas (popns. 12, 13, 26) from the central Texas and northward populations, in both the terpenes and morphology (Fig. 2). Notice that populations 12 (Ozona, TX), 13 (Comstock, TX), 25 (Pandale, TX) and 26 (Coahuila, MX) are divergent in both their essential oils (Fig. 2, left) and morphology (Fig. 2, right). In addition, population 17 (nw of New Braunfels, TX) is somewhat divergent in its essential oil, but less so in its morphology (Fig. 2, right). It is interesting to note that all the other populations of *J. ashei*, even those in isolated areas, show almost no variation in either the leaf oils or the morphology. The Trans-Pecos, Texas and Mexico populations

(ancestral or pre-Pleistocene) have more elliptical whip leaf glands, whereas the balance of *J. ashei* populations (Holocene or post-

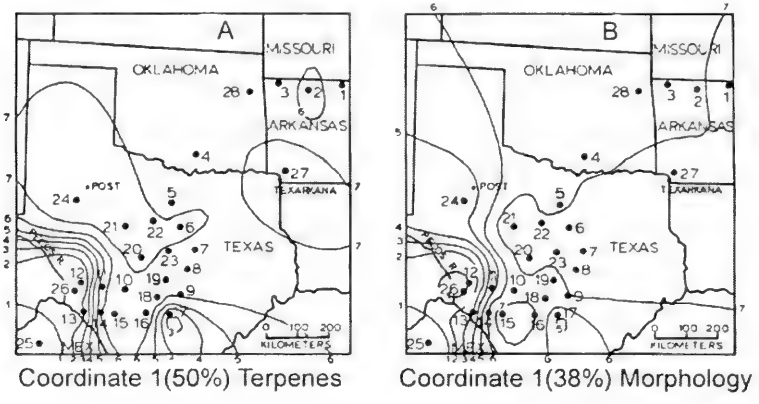

Figure 2. Principal coordinate analyses (PCO) of terpenoids (A) and morphology (B) of *J. ashei*. Adapted from Adams, 1977.

Pleistocene) have hemispherical glands that are unique to *Juniperus* (Adams, 2004). The hemispherical glands seem to be a derived condition in *Juniperus*.

Although there is considerable evidence of a continuous band of sclerophyllous vegetation from central Texas into northern Mexico during the Tertiary (Axelrod, 1975), it is more productive to focus on events in the Pleistocene, particularly the last pluvial and interglacial periods. According to King (1973), the western Ozarks were covered with boreal spruce forest from about 25,000 to at least 13,000 B.P., with pine parkland preceding the boreal spruce forest. The pine parkland and boreal spruce forest both appeared to have been pushed southward from the north (Dillon, 1956). Figure 3 shows the hypothetical vegetation during the pluvial period (modified from Adams, 1977). The area south of the Ozarks may have been pine woodland or parkland (see Bryant, 1969). A pine-spruce woodland seems likely on the Llano Estacado of northwest Texas according to Hafsten (1961). Bryant (1969) suggested that, based on pollen profiles, the present Chihuahuan desert area around Del Rio, TX (430 m) was pinyon woodland. Wells (1966), using data obtained from rat middens from the Big Bend region of Texas, concluded that life zones

descended about 800 m for pinyon-juniper (*J. pinchotii* in that case), allowing the advance of pinyon-juniper into most of the present desert region between the Big Bend and

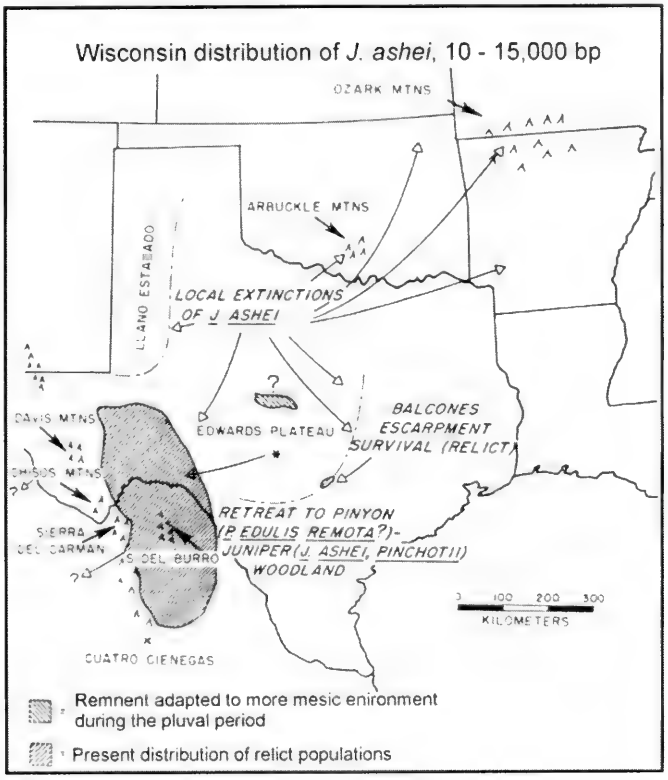

Figure 3. Possible *J. ashei* distribution during the Wisconsin era (from Adams, 1977, 2004).

Del Rio. Typical *J. pinchotii*, and the ancestral (Pleistocene) type, *J. ashei,* have been found growing just south of the Sierra de Carmen mountains of the Big Bend region (Adams, 2004). It appears that the Sierranas del Burro, Mexico may have been an important refugium or "island point" in the pinyon-juniper woodland. A mixed deciduous woodland with conifers is postulated in central Texas (Bryant, 1969) based on pollen profiles.

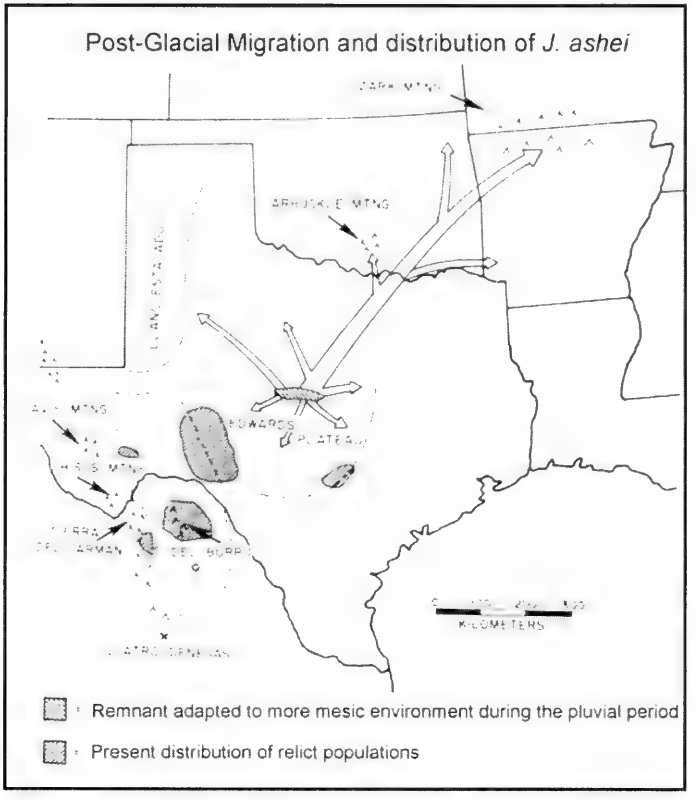

Figure 4. Postulated post-glacial recolonization of *J. ashei* onto limestone producing very uniform populations (Adams, 1977, 2004).

At the end of the Wisconsin glacial advance (10,000 - 13,000 yr bp), the central Texas, Oklahoma and Arkansas populations of *J. ashei* were likely extinct because this area was a much wetter and cooler spruce woodland (Fig. 3).

Adams (1977, 2004) postulated that *J. ashei* was pushed south into refugia in west Texas and Mexico (Fig. 3). It appears that the ancestral (Pleistocene) *J. ashei* was lower in camphor and had somewhat elongated whip leaf glands. The more recently derived (Holocene) populations (Fig. 4) are higher in camphor and have whip leaves with

hemispherical oil glands that are unique within *Juniperus* (Adams, 2004).

During the same period, *J. ashei* may have expanded south and west into the Chihuahuan desert (Wells, 1966), but not as far south as Cuatro Cienegas, Coahuila, Mexico (Meyer, 1973). Migration of populations to regions west of the Sierra del Carmen was also possible because *J. ashei* grows at the top of La Cuesta pass just south of the Sierra del Carmen (Adams, 1977). With this model, populations of *J. ashei* were forced to extinction in central Texas, Oklahoma, Arkansas, and Missouri. The subsequent recolonization in the Holocene could then take place as depicted in figure 4, over a very short time from a relictual population in central Texas that may have gone through a selection 'bottleneck' perhaps coupled with genetic drift. This 'relict' population would have had considerably more camphor in the oil, more hemispherical glands, larger female cones, fewer seeds (therefore a higher pulp to seed ratio as a reward for birds), and a more lax foliage which seems to be associated with more mesic junipers. The rapid colonization of limestone outcrops (Fig. 4) could then lead to a uniform taxon from central Texas to the Ozarks. In addition, this Holocene type of *J. ashei*, was competitive in invading grasslands that still flourished in the post-Wisconsin pluvial period (Adams et al. 1998a). The pre-Pleistocene (ancestral) *J. ashei* is restricted to drier, rocky habitat in far west Texas and northern Mexico.

The purpose of this paper is to present new analyses of the leaf oils and DNA fingerprinting (RAPDs) of the wide-spread *J. ashei* from the Edwards Plateau, and *J. ashei* from the semi-arid area around Ozona, TX, and to re-evaluate the taxonomic status of the divergent, pre-Pleistocene populations.

MATERIALS AND METHODS

Specimens used in this study: *Juniperus ashei,* Coryell Co., TX, *Adams 7424-42* 2 km se of jct. of CR314 and FR107 on CR314; Crockett Co., TX, *Adams 7463-82*, 5 km w of Ozona, on US290. Voucher specimens are deposited at Baylor University (BAYLU).

Fresh leaves (200 g. fresh wt.) were steam distilled for 2 h using a circulatory Clevenger apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored

at -20°C until analyzed. The extracted leaves were oven dried (48h, 100°C) for determination of their oil yields.

The essential oils were analyzed on a HP5971 MSD mass spectrometer, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2006 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2006), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by TIC.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5'-3'): 134, AAC ACA CGA G; 153, GAG TCA CGA G; 204, TTC GGG CCG T; 218, CTC AGC CCA G; 227, CTA GAG GTC C; 236, ATC GTA CGT G; 239, CTG AAG CGG A; 244, CAG CCA ACC G; 250, CGA CAG TCC C; 265, CAG CTG TTC A; 268, AGG CCG CTT A; 338, CTG TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G.

PCR stock solutions (Taq, primer, buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams et al. 1998a, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams et al. 1998a). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating/ cooling block. The thermal cycle used was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 40°C (2 min), 72°C (2 min), 91°C (1

min). Two additional steps were used: 40°C (2 min) and 72°C (5 min) for final extension. The temperature inside a PCR tube containing 15 µl buffer was monitored with a temperature probe, quantitated and printed for each step for each of the 40 cycles for every PCR run (Adams et al.1998a) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were photographed over UV light using Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring. Bands were scored as present (1) and absent (0). Bands that were inconsistent in replicate analyses were not scored.

Associational measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principle coordinate analysis (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). It should be noted that problems of homology of RAPD DNA bands on agarose gels can be significant (Rieseberg, 1996), but these errors can be accounted for using multivariate statistical methods (PCO) (see Adams and Rieseberg, 1998). A minimum spanning diagram was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes (Adams et al. 2003).

RESULTS AND DISCUSSION

Comparing the leaf essential oils semi-arid (ancestral) versus more mesic, Edwards plateau junipers revealed that they differ mostly in a quantitative fashion (Table 2). Camphor is considerably larger (Table 2) in more recent populations (69.1%) than in ancestral populations (53.5%). In contrast, bornyl acetate is must larger in ancestral (15.6%) than in recent (6.3%) populations (Table 2). Four (non-trace) compounds differ qualitatively: trans-sabinene hydrate, trans-p-menth-2-en-1-ol, verbenone, and sandaracopimara-8(14),15-diene (Table 2). Several other compounds differ quantitatively: α-pinene, myrcene, p-

cymene, limonene, γ-terpinene, linalool, trans carveol, carvone and elemol (Table 2).

Principal Coordinate Analysis (PCO) using 175 RAPD bands resulted in the complete separation of the ancestral (Ozona) from the recent (Waco, Edwards plateau) plants (Fig. 5). The first coordinate

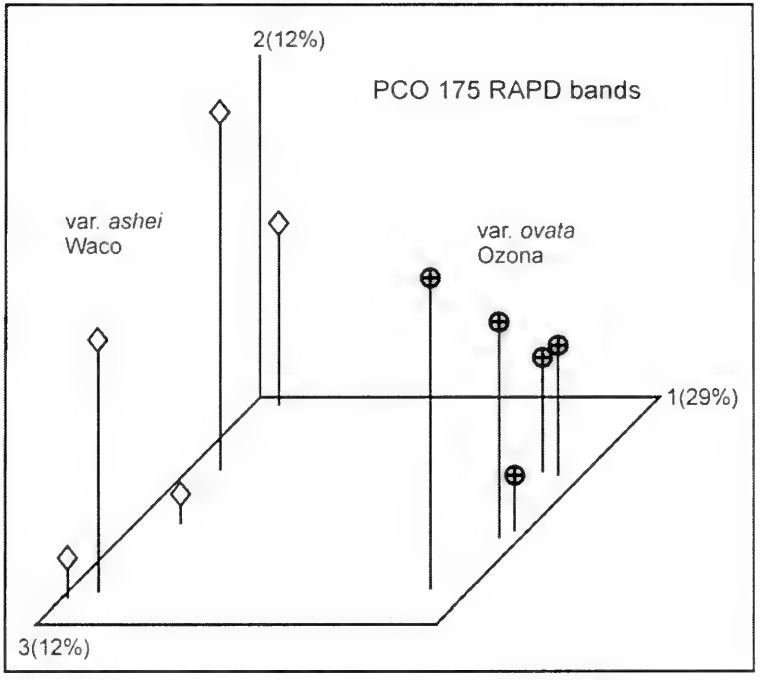

Figure 5. PCO of *J. ashei* based on 175 RAPD bands.

removed 29% of the variance among individuals and clearly shows that the populations are distinct in their DNA.

Type material of *J. ashei* consisted of one male and three female specimens (Hall, 1954). Because of this, Hall (1954) selected a female specimen (acc. number *22520*, 16 Sept. 1923, UNC) and designated it as the lectotype. All of the material cited by Buchholz (1930) was collected on limestone bluffs, above the White River, near Sylamore, Arkansas. It is clear from the figure in Buchholz (fig. 1,) that his

specimens had hemispherical glands on the whip leaves were representative of the post-Pleistocene *J. ashei.*

Based on the differences in morphology, leaf essential oils and DNA, there is sufficient differentiation to recognize the ancestral, pre-Pleistocene populations from far west Texas and Coahuila, Mexico as a distinct variety:

Juniperus ashei var. ***ovata*** R. P. Adams, **var. nov**. TYPE: U. S. A., Texas, Crockett Co., 5 km w. Ozona, 6 Dec. 1994, *R. P. Adams 7463* (HOLOTYPE: BAYLU, PARATYPES: *R. P. ADAMS 7664, 7465, 7466, 7467* (BAYLU).

Junipero ashei var. ashei similis sed differt flagellifoliis glandibus elevatis ovalibus vel elongatis, strobilis minoribus, et seminibus in quoque strobilo plus numerosis.

The new variety is like *Juniperus ashei* var. *ashei*, but instead of hemispherical glands, the glands are oval to elongated on the whip leaves. *Juniperus ashei* var. *ovata* also has smaller cones, and more seeds per cone than *J. a.* var. *ashei.*

Other specimens examined: MEXICO, Coahuila, *Adams 1066-1076.* U.S.A., Texas, Crockett Co., Ozona, *Adams 7424-42* (BAYLU), Coryell Co., TX, *Adams 7463-82* (BAYLU!).

Key to *J. ashei* varieties:

1. Glands on whip leaves hemispheric; female cones (8) 9 (10) mm in diameter; seeds 16-27 mm^2, 1 (rarely 2, avg. 1.01) per cone .....*J. ashei* var. *ashei*
1. Glands on whip leaves oval to elliptical; female cones (5) 6 (8) mm diam.; seeds 13-16 mm^2, 2 (avg. 1.7), per cone .....*J. ashei* var. *ovata*

The whip leaf glands are illustrated in figure 5. Notice hemispherical glands on var. *ashei* (Fig. 6, left) and the raised, oval to elongated glands on var. *ovata* (Fig. 6, right). It should be noted that a few nearly hemispherical glands are present on whip leaves of var. *ovata.* This is informative, as these characters can be used to distinguish var. *ovata* from var. *ashei*, yet exclude other nearby juniper

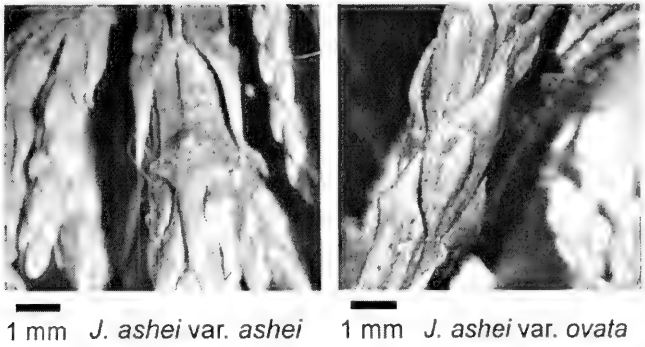

Figure 6. Comparison of whip leaf glands for *J. ashei* var. *ashei* and *J. a.* var. *ovata*.

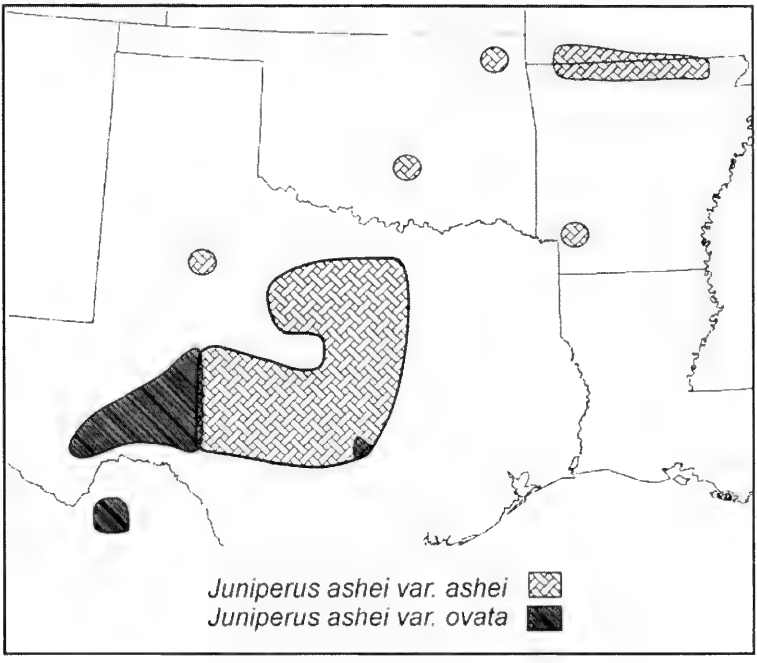

Figure 7. Distribution of *J. ashei* var. *ashei* and *J. a.* var. *ovata*.

species such as *J. monosperma* (Englem.) Sarg. *J. pinchotii* Sudw. and *J. coahuilensis*. (Mart.) Gaussen ex R. P. Adams.

The distribution of the two varieties is shown in fig. 7. The area of possible sympatry in west Texas and around New Braunfels is not well understood and additional field collections are needed to define better their distributions in these areas.

ACKNOWLEDGEMENTS

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Table 1. Differences between *J. ashei* var. *ashei* (recent, post-Pleistocene) and *J. a.* var. *ovata* (ancestral, Pleistocene relicts).

Character	*J. a.* var. *ashei*	*J. a.* var. *ovata*
female cone diameter	larger (8)9(10) mm	smaller (5)6(8)mm
seeds per cone	fewer (1.01)	more (1.7)
seed size (L x W)	larger (16-27 mm^2)	smaller (13-16 mm^2)
whip leaf gland length/ sheath length	smaller ratio (0.20-0.30)	larger ratio (> 0.40)
whip leaf gland shape	hemispherical (1.0 - 1.5)	raised, oval to ellipse (2.0 - 2.5)
branching angle	narrow (45 - 40°)	wider (45 - 55°)

Table 2. Comparisons of the per cent total oil for the leaf essential oils of *J. ashei* var. *ashei* and *J. a.* var. *ovata*. Large differences in concentrations are highlighted in boldface.

RI	Compound	var. *ashei*	var. *ovata*
921	tricyclene	1.3	1.1
933	**α-pinene**	**0.4**	**3.8**
946	camphene	1.6	1.6
969	sabinene	t	0.3
974	β-pinene	t	-
988	**myrcene**	**0.5**	**2.6**
1001	δ-2-carene	t	-
1002	α-phellandrene	t	t
1008	δ-3-carene	t	0.1
1014	α-terpinene	t	t
1020	**p-cymene**	**2.0**	**0.7**
1024	**limonene**	**3.5**	**7.7**
1025	β-phellandrene	t	t
1054	**γ-terpinene**	**0.2**	**0.8**
1067	cis-linalool oxide (furanoid)	t	-
1078	camphenilone	t	-
1084	trans-linalool oxide (furanoid)	0.3	0.4
1086	terpinolene	t	t
1095	**linalool**	**1.4**	**0.4**
1098	**trans-sabinene hydrate**	**0.2**	**-**
1100	isopentyl 2-methyl butanoate	t	-
1112	3-methyl butanoate, 3-methyl-3-butenyl-	t	t
1118	cis-p-menth-2-en-1-ol	t	t
1122	α-campholenal	t	-
1136	**trans-p-menth-2-en-1-ol**	**0.2**	**-**
1141	**camphor**	**69.1**	**53.3**
1145	camphene hydrate	0.3	0.3
1165	borneol	2.2	2.8
1174	terpinen-4-ol	0.3	0.5
1179	p-cymen-8-ol	0.3	0.1
1186	α-terpineol	0.1	t

Table 2, continued.

RI	Compound	var. *ashei*	var. *ovata*
1204	**verbenone**	**0.1**	-
1207	trans-piperitol	0.2	t
1215	**trans-carveol**	**0.7**	**t**
1218	endo-fenchyl acetate	t	-
1226	cis-carveol	t	t
1239	**carvone**	**0.8**	**t**
1249	piperitone	t	-
1273	trans-carvone oxide	t	-
1287	**bornyl acetate**	**6.3**	**15.6**
1289	p-cymen-7-ol	t	-
1298	carvacrol	t	-
1339	trans-carvyl acetate	t	t
1340	piperitenone	t	-
1548	**elemol**	**0.2**	**0.9**
1649	β-eudesmol	t	0.4
1652	α-eudesmol	t	0.5
1968	**sandaracopimara-8(14),15 -diene**	**0.2**	-
1987	manoyl oxide	3.6	3.2
2055	abietatriene	0.2	0.2
2087	abietadiene	0.2	0.3
2282	sempervirol	1.1	0.5
2314	trans-totarol	0.7	0.3
2331	trans-ferruginol	0.2	0.1

0.05% are denoted as traces (t). Unidentified components less than 0.5% are not reported. RI is the arithmetic retention index in DB-5.

FACTORS THAT INFLUENCE THE DISTRIBUTION AND COVER OF *HELIANTHUS PARADOXUS* IN A WEST TEXAS SALT MARSH

Matthew Grunstra
Department of Earth and Environmental Science
University of Texas at San Antonio
San Antonio, Texas, USA, 78249-0663
matthew.grunstra@utsa.edu

O. W. Van Auken
Department of Biology
University of Texas at San Antonio
San Antonio, Texas, USA, 78249-0663
oscar.vanauken@utsa.edu

ABSTRACT

The Diamond Y Spring is one of the last major flowing springs in west Texas. It is habitat for six federally endangered or threatened species including *Helianthus paradoxus*, the Pecos (= puzzle) sunflower. While the plant communities in the Diamond Y Spring Preserve seem to be fairly well delineated, the *H. paradoxus* population and distribution varies considerably on an annual basis. Some relationships between *H. paradoxus* growth and some abiotic factors including soil moisture, soil salinity, and soil oxygen have been examined in other studies. This study uses GIS mapping techniques to investigate relationships between *H. paradoxus* cover and the abiotic soil characteristics. Locations in the marsh with *H. paradoxus* at greater than 5 % cover were found to coincide with locations with depth to water greater than 25 cm, soil salinity between 7 and 12 g/kg, and soil pH between 8.3 and 8.5. This study suggests that annual fluctuations of the cover and distribution of the *H. paradoxus* populations is influenced by the level of water coupled with soil pH and soil salinity found in the salt marsh.

KEY WORDS: *Helianthus paradoxus*, sunflowers, soil salinity, arid environments, inland salt marshes, spatial changes, distribution

The genus *Helianthus* is composed of approximately 67 annual and perennial species divided into four sections (Correll and Johnston 1979; Heiser 1965). *Helianthus paradoxus* is an annual hybrid species belonging to the Asteraceae family and was proposed for listing as a federally threatened species in 1998 and listed in 1999 (McDonald 1999). *Helianthus paradoxus* and its parental species *H. annuus* and *H. petiolaris,* all belong to the same section with the same chromosome number (n = 17) (Rieseberg 1991; Rieseberg et al. 1990). The species are easily delineated based on distinct morphological, phenological and habitat characteristics (Correll and Johnston 1979; Heiser 1958; McDonald 1999).

Helianthus paradoxus differs from *H. annuus* in having narrower leaves, fewer hairs on the stems and leaves, smaller flower heads, narrower less abruptly acuminate phyllaries, and later flowering (Heiser 1958). It differs from *H. petiolaris* in having shorter petioles and no hairs at the tips of the pales of the flower head (Heiser 1958). *Helianthus paradoxus* is intermediate between its parents in morphology, but not in habitat preference likely indicating a long period of independent evolution after its origin (Rieseberg et al. 1990; Schilling and Heiser 1981). Molecular studies have indicated *H. paradoxus* is a true species and estimated the hybridization from *H. annuus* and *H. petiolaris* occurred approx. 75,000 to 208,000 years ago (Rieseberg et al. 1990; Rieseberg 1991; Welch and Rieseberg 2002).

Helianthus paradoxus is able to establish and persist in an extreme habitat, an inland sulfate dominated salt marsh. Neither parental species grow in the marsh nor are they competitive under similar salinities (Bush and Van Auken 2004; McDonald 1999; Welch and Rieseberg 2002; Van Auken and Bush 2006). *Helianthus paradoxus* germinates in January when soil water content is highest and surface salinity is lowest (Van Auken 2001). *Helianthus paradoxus* seems to be a true wetland species that is usually found in saturated saline soils and will continue to grow even when inundated although at reduced levels (Bush 2006a).

Saline habitats are quite often thought of as existing near large bodies of saltwater. While those environs account for most of the worlds salt marsh habitat, inland salt marshes occur in many different

geographical areas worldwide (Odum 1988). Inland salt marshes can develop within high-evaporation basins, next to small inland saline lakes, and lowlands associated with desert springs (Odum 1988). Often small inland salt marshes are found in desert areas without a large body of water nearby. Inland salt marshes occur throughout the southwestern desert areas of North America due to the high rate of evaporation and low and highly variable level of precipitation in the desert ecosystems (Shaw and Fredine 1956). The level of salts and the kind of salts found across an inland salt marsh vary in both time and location (Borchert 1971; Ungar 1974). This phenomenon is due to the variable amount of precipitation in the system and the underlying substrate (Brune 1981).

Limited tolerance of most plant species to salt damage typically accounts for saline habitats having low species diversity (Chapman 1974). Sodium (Na^+) and chloride (Cl^-) can be extremely toxic to most plants at moderate to high concentrations (Lavelle and Spain 2001). Relatively few plant species have evolved structural, physiological, and/or biochemical mechanisms of salt resistance (Salisbury and Ross 1991; Troughton and Donaldson 1972).

The locations of the specific plant communities in salt marshes have been suggested to depend mainly on the differing species tolerances to the varying abiotic factors (Bush 2002; Chapman 1974; Cooper 1982; DeJong 1978; Ewing 2000; Grunstra and Van Auken 2006; Naidoo et al. 1992; Rand 2000; Snow and Vince 1984; Van Auken and Bush 2006). Four major plant species including *Helianthus paradoxus*, *Sporobolus airoides* (alkali sacaton), *Schoenoplectus americanus* (formerly *Scirpus americanus*, bulrush), and *Distichlis spicata* (saltgrass) as well as several other minor species inhabit the salt marsh of the Diamond Y Spring Preserve (Grunstra and Van Auken 2006; Van Auken and Bush, 1998; Van Auken 1998; Van Auken and Bush 2006; Van Auken et al. 2006) (Figure 1). The locations of the salt marsh species are most likely due to their water requirements, salt tolerance, or ability to out-compete rivals in the differing salinity levels of the soil (Bertness 1991; Bush 2006b; Chapman 1974; Niering and Warren 1980; Van Auken and Bush 1998).

Some studies have used statistical techniques to identify the role of the abiotic factors present on the distributions of the species in a salt

marsh area. Bush (2006a,b) used regression techniques to show that salinity and the soil moisture content played an important role in the growth of *H. paradoxus*. Dependence on soil salinity and soil moisture was also shown in an analysis of the plant communities of an Egyptian inland salt marsh (El-Ghani 2000).

Figure 1. Example of the delineation of the plant species into distinct areas across the marsh landscape in the Diamond Y Spring Preserve located in Pecos County, Texas. The species present in the photograph are mainly: *Sporobolus airoides* in the foreground, *Distichlis spicata* is at midfield, and *Schoenoplectus americanus* is the dark vegetation across the middle-top of the picture.

The purpose of this study was to determine and map the abiotic factors of water table depth, soil pH and soil salinity in the salt marsh of the Diamond Y Spring Preserve through one growing season, as well as estimating the plant cover of *D. spicata*, *H. paradoxus*, *S. americanus*, and *S. airoides*. Geographical Information System (GIS) software was used to show the relationship between locations in the salt marsh maintaining specific levels of water table depth, soil pH, and soil

salinity with the locations identified as high in *H. paradoxus* cover. These abiotic levels most likely play an important role in determining the growth and distribution of *H. paradoxus* in the salt marsh of the Diamond Y Spring Preserve.

METHODS

The Diamond Y Spring Preserve is located in Pecos County Texas and occupies a total area of approximately 6.1 km^2. The study site encompasses the 37 ha salt marsh formed near the junction of the Diamond Y Spring and Leon Creek drainages. Historically, this is one of the main areas in which the federally threatened *H. paradoxus* grows (Bush 2002; Van Auken and Bush 1998). The borders of the study area are limited on the western and eastern edges by fencing (Figure 2). The northern and southern limits of the study area are delimited by limestone outcroppings producing a sharp change in elevation out of the lowland salt marsh and into slightly higher *Prosopis glandulosa* (honey mesquite) and *Larrea tridentata* (creosote bush) woodlands.

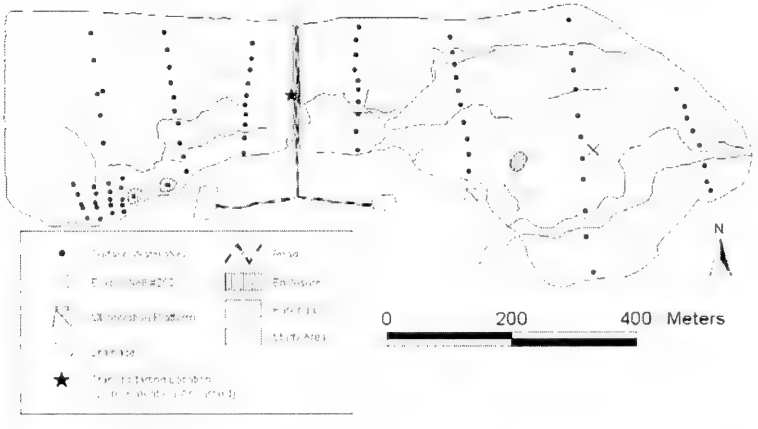

Figure 2. Overview of the 37 ha study area and the observation site grid pattern. The locations of the 87 observation sites and surface features in the study area are labeled. The dirt roadway crossing the marsh and connecting the two observation parking lots is shown. The observation platform can be seen on the southern edge of the eastern side of the study area.

The water table for the salt marsh was studied using shallow piezometers or observation wells based on a design used in previous studies in the marsh area as well as standard monitoring well construction techniques (Bush 2002; EPA 1975; WRP 1993). The piezometers consisted of 5 cm in diameter PVC pipe buried vertically to a depth of approximately 60 cm in the ground. A grid pattern was planned based on the existing pattern of wells located in the southwestern corner of the marsh (Figure 2). The grid pattern was expanded to a larger scale to increase the size of the study area and encompass more of the lowland region of the salt marsh, especially areas where *H. paradoxus* populations were previously found (Van Auken and Bush 1998). The well site locations, as well as, many surface features of the study area were mapped using Trimble's GeoExplorer III GPS and Beacon-on-a Belt units (Figure 2).

Water table depths were monitored at the 87 piezometers in the study area and recorded on a monthly basis beginning in January 2002 and continuing until October 2002 for 870 total observations. This time period included observations in both the wet and dry seasons of the marsh area as well as covering the growing season of the marsh plants including *H. paradoxus* (Bush 2002; McDonald 1999).

In addition to water depth, soil samples were collected at each well site. A total of 870 samples were collected, eighty-seven during each monthly collection. Approximately 300 g of surface soil was gathered at a 2 m distance in a cross pattern around each well pipe. Surface litter was removed and the sample was collected from the top 1 cm of soil (TAES 1983). After the soil was air-dried, the sample was crushed and sieved with a USDA size 4 mesh to remove debris and rock fragments. Salinity and pH of the soil were measured by making a 1:1 paste (soil:de-ionized water) then measuring with a pH meter and salinity probe. (Rowell 1994; TAES 1983; Westerman 1990). This data was then entered into the ArcView 3.3 GIS software.

During the month of October 2002, estimations of the percent plant cover for the major herbaceous species were made across the study area. October was chosen in order to observe *H. paradoxus* in bloom (McDonald 1999). A 1.0 m^2 (1 m x 1 m) quadrat was located 1.5 m to the west of each well location. Locating the quadrat 1.5 m to the west,

removed any possible effect from the disturbance caused by digging the well or any subsequent foot travel in the area of the well.

The point data sets of the depth to water, pH, and soil salinity were converted to a raster grid to produce a continuous surface or contour plot across the study area.

This analysis was done by calculating the mean values for each of the well site locations from the ten-month data sets for each of the factors (depth to water, soil pH, and soil salinity). Surface contour plots were then created for the mean values of the factors using the IDW interpolation method available in the Spatial Analyst extension of the ArcView 3.3 GIS software. These surface contour plots were then examined using the map calculator function in the Spatial Analyst extension (ESRI 1999; Ormsby and Alvi 1999).

The map calculator was used to identify the locations of greater than 5 percent *H. paradoxus* cover in the study area. The surface contour maps were then systematically evaluated for values corresponding to the locations of the high *H. paradoxus* cover by beginning with large intervals of acceptance for the three different factors and slowly reducing the intervals until an area was left matching the high *H. paradoxus* cover locations.

RESULTS

Helianthus paradoxus cover was low during this study (Figure 3, top). There were few *H. paradoxus* plants found in the study area. A small concentration of *H. paradoxus* with a high total coverage was found in the southwestern portion of the study area (Figure 3, top). The eastern half of the study area had a couple of small communities of *H. paradoxus* but all had a low percentage of coverage with only one location over 5 % total coverage (Figure 3, top). Across the marsh single *H. paradoxus* plants could be seen but were not usually located and represented in the sampling. Using the map calculator feature of ArcView 3.3 the study area was searched for high densities (greater than 5 %) of *H. paradoxus*. The light gray areas indicate such areas (Figure 3, bottom).

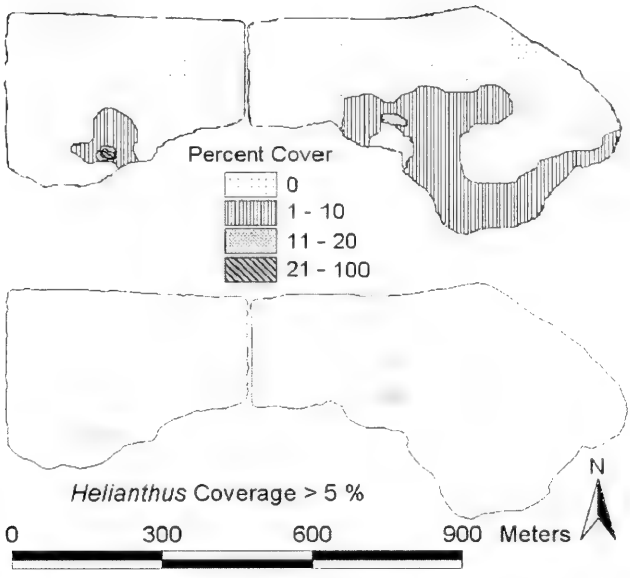

Figure 3. Top - Surface contour plots of the ten-month *Helianthus paradoxus* mean cover created using the IDW interpolation method in the ArcView 3.3 GIS software. Bottom - Surface plot created using the map calculator feature of ArcView 3.3. The calculator was set to search for areas with *H. paradoxus* cover greater than 5%. The light gray colored areas are where the search returned a positive result.

The ten-month averages of the abiotic soil factors were created to show the trends of the three abiotic factors (Figure 4). The mean depth to water was found to be relatively shallow (0 - 10 cm) across 68 % of the study area (Figure 4, top). There were several locations with standing water. The average depth to water increased from the center of the marsh and/or locations of the drainage areas towards the northern and southern borders of the study area (Figure 4, top). This coincided with the elevation and slope increasing towards the hills and limestone outcroppings in the northern and southern directions.

Most of the study area was found to have low levels of salinity between 3 - 10 g/kg (Figure 4, middle). High levels were found along

the northern and southern boundaries with the highest levels concentrated on the western side of the marsh. These areas tended to be of higher elevation and away from the lower areas and drainages. The highest average values were in the range of 26 - 43 g/kg for salinity (Figure 4, middle).

The averages of the soil pH levels across the marsh revealed the largest portion of the study area was in the pH range of 8.2 - 8.4 (Figure 4, middle). Soils with an average in the 7.5 - 8.1 pH range occupied the center or lower elevation of the marsh. These lower pH areas tended

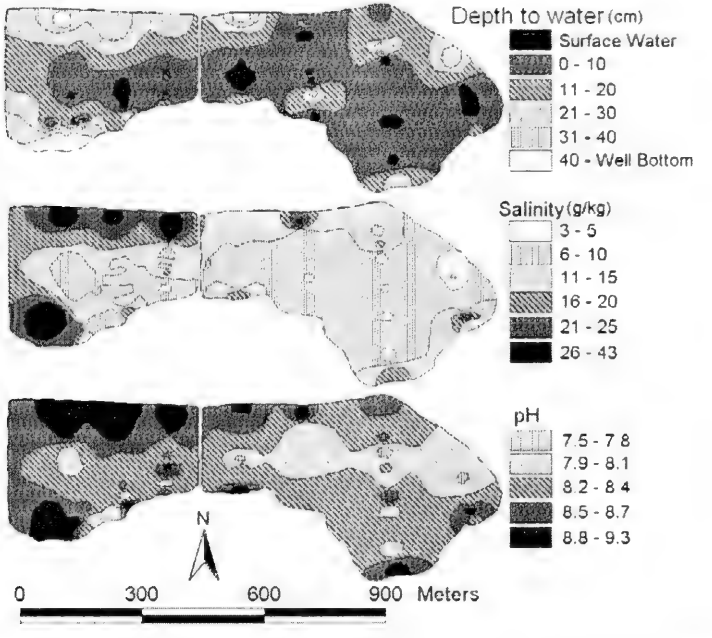

Figure 4. Surface contour plots of the ten-month abiotic factor means created using the IDW interpolation method in the ArcView 3.3 GIS software. Top plot shows the ten-month average depth to water (cm). The middle plot shows the ten-month average soil salinity across the marsh (g/kg). The bottom plot shows the salt marsh's ten-month average soil pH.

to correspond to the areas of the marsh with an average higher water table (Figure 4, top and bottom). High average pH values were found along the northern and southern border specifically on the western half of the study area (Figure 4, bottom). These high soil pH areas had average values between 8.8 and 9.3 and corresponded to areas with a deeper water table (Figures 4, top and bottom).

The ten-month averages of the abiotic soil factors (depth to water, salinity, pH) were examined at locations of the *H. paradoxus* cover (Figure 5). Individually the three characteristics did not seem to influence where high *H. paradoxus* cover areas were located. The three factors by themselves (alone) did not directly correspond to the high *H.*

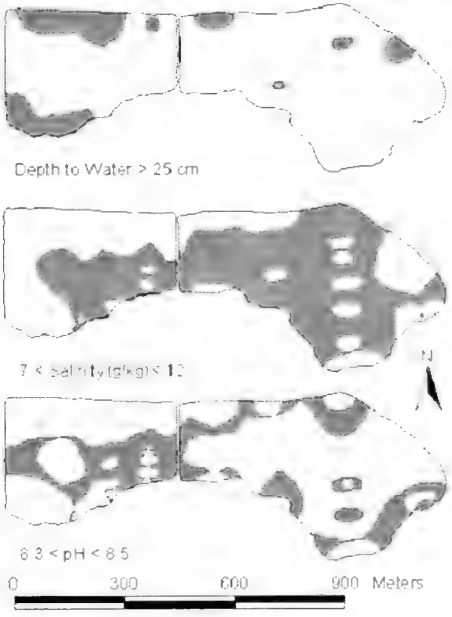

Figure 5. Analysis plots showing search conditions and locations (shaded areas) lying within those conditions. Top plot indicates where the average depth to water is greater than 25 cm. The middle plot specifies locations with an average of salinity levels between 7 and 12 g/kg. The bottom plot identifies areas with an average pH level between 8.3 and 8.5.

paradoxus cover areas (Figure 5). The factors were then combined to determine possible combinations that would correspond to the high *H. paradoxus* cover locations. Combinations of depth to water and soil salinity, depth to water and soil pH, and soil salinity and soil pH did not correspond to the high *H. paradoxus* cover locations. However, when the three factors were combined and the following search criteria were used: depth to water greater than 25 cm, soil salinity between 7 and 12 g/kg, and pH between 8.3 and 8.5, an interesting relationship emerged (Figure 6). The only areas in the salt marsh that met these three conditions were the same locations as the 5 % *H. paradoxus* cover (Figure 6). No other combinations of the factors examined matched the 5 % *H. paradoxus* cover distribution.

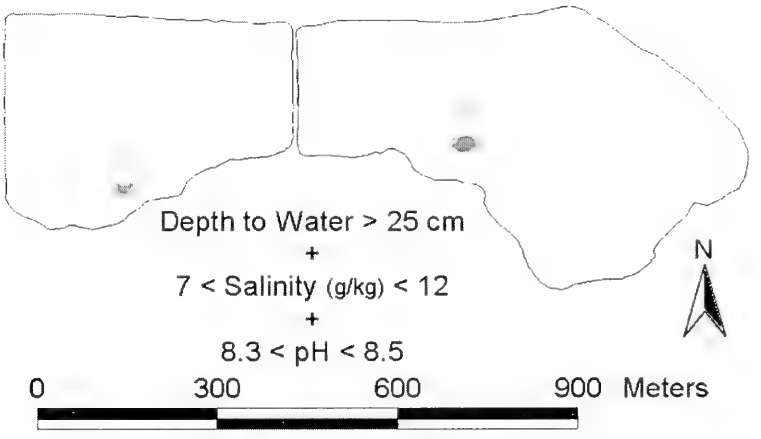

Figure 6. Surface plot created from aligning the *H. paradoxus* search criteria with the combined search criteria (depth to water greater than 25 cm, salinity between 7 and 12 g/kg, and pH between 8.3 and 8.5). Positive results for the combined search are shown in the dark color. The lighter gray color areas are the locations with greater than 5 % *H. paradoxus* cover. The *H. paradoxus* location and search criteria locations are overlapping.

DISCUSSION

Historically, high numbers of *H. paradoxus* plants have been found in the salt marsh of the Diamond Y Spring Preserve. Usually with so many plants present in October the sunflowers cover the study area in a sea of yellow flowers (Van Auken 2002). During the 2002 study period, the *H. paradoxus* plants were only found sporadically across the study area (Figure 3). The low density of this species was most likely due to the atypical rainfall and soil water pattern of that year.

The rainfall pattern for 2001-2002 was not anything like the long term monthly mean for the area (Figure 7) (NCDC 2002). January of the study year received no precipitation when it usually receives approximately 2 cm while June and July received greater amounts of precipitation than expected (Figure 7). The month of August shows very little precipitation (0.4 cm) compared to the mean precipitation

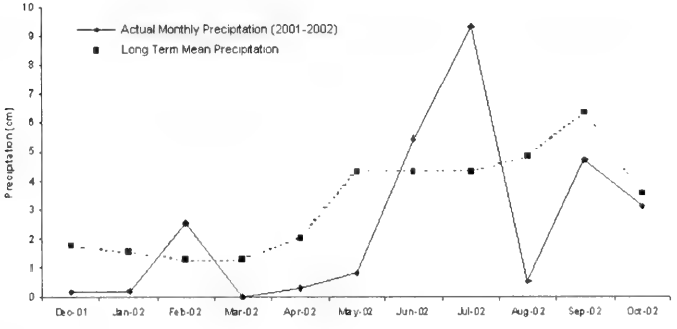

Figure 7. Total monthly precipitation data from December 2001 to October 2002. The values are plotted for the center of the months. The solid line is the actual monthly precipitation reported from Fort Stockton, Texas. The mean precipitation data is based on 1231 months from years 1859-1987 (NCDC, 2002).

normally expected during that month (5 cm) (Figure 7). The variation from the annual mean precipitation had a pronounced effect on the level of moisture in the marsh. In July of 2001 when precipitation followed a

mean trend, only 0.5% of the study site had surface or subsurface water between 0 and 10 cm below the surface while for July 2002 almost 60% of the study area had water between 0 and 10 cm below the surface (Grunstra 2002).

The locations of the mean water table depths can be readily seen in the surface contour plots created in this study (Figure 4, top). The shallow surface water areas corresponded to the central lower elevation areas of the salt marsh while the upper elevations had little to no surface water and a much deeper water table on average. This central low area of the marsh also maintained the lowest levels of soil pH and soil salinity throughout the growing season (Figure 4). The higher elevations in the study area consistently showed the highest levels of soil pH and soil salinity (Figure 4, middle and bottom).

In the Diamond Y salt marsh, the higher salinity and pH values at the northern and southern borders are attributed to a deeper water table that allows the surface soil to dry and deposit higher levels of soil surface salt. The areas with high soil salinity have been shown to grow larger seasonally as the water table got deeper the surface and marsh dried out (Grunstra 2002; Grunstra and Van Auken 2006). The same high surface soil salinity areas then receded when the water table rose and the salts were most likely flushed from the surface soil by the surface water.

The spatial patterns and trends of different abiotic factors as well as their interactions may play an important and significant role in the distribution of the salt marsh vegetation. It has been suggested that the *H. paradoxus* communities tend to move around the study area from year to year possibly caused by soil water content, salinity, temperature and the interaction of these factors (Grunstra and Van Auken 2006; Van Auken and Bush 1993; Van Auken and Bush 1995). Spatial distributions of soil moisture, pH, and ionic composition were found to be significant in determining plant community locations in a Mediterranean salt marsh (Rogel et al. 2001). In an inland salt marsh within the Great Basin Desert of Utah, spatial succession has been shown to be related to the plant species salinity tolerance (Bolen 1964). Bush (2002) found that surface salinity had a negative effect on all growth parameters and aboveground dry mass of *H. paradoxus* at the

Diamond Y Spring Preserve depending on the time of year. Several studies based on vegetation communities of coastal marshes have indicated the importance of soil salinity and community distribution (Bertness 1991; Naidoo et al. 1992; Ewing 2000; Rand 2000).

Based on the results from this study and disregarding possible biotic relationships, *H. paradoxus* appears to have a niche in areas where the average depth to water is greater than 25 cm, the average soil salinity level is between 7 and 12 g/kg, and the average soil pH is between 8.3 and 8.5. These findings are in agreement with other studies which found *Helianthus paradoxus* to be restricted to areas with surface salinity levels of approximately 10 g/kg (Bush 2006b; Poole 1992; Poole and Diamond 1993; Siviniski 1996). Bush (2006b) further showed that the abiotic factor which best determined dry mass of *H. paradoxus* was determined by the time of year. In that study, regression analysis indicated that soil salinity was the most important determinant of *H. paradoxus* above ground dry mass, except later in the growing season when surface moisture was the most important factor.

In a growth box experiment, soil moisture was found to be the most important factor regarding *H. paradoxus* growth (Bush 2006a). In the same experiment, the higher soil salinities were also shown to inhibit *H. paradoxus* growth. The salinity levels showing reduced growth by Bush were much lower than some of the salinity levels found to occur in the salt marsh. This may indicate that in the areas of higher salinity levels found in the salt marsh *H. paradoxus* may experience reduced growth or be prevented from establishment in those locations entirely.

Although abiotic factors have been determined to be important, biotic factors such as competition may also play a role in *H. paradoxus* distribution (Bertness 1991). Previous competition experiments have given varied results. Field experiments with *Sporobolus airoides* and *Distichlis spicata* have been shown not to inhibit *H. paradoxus* establishment (Jackson 2001; Van Auken and Bush 2006). However, competition from *D. spicata* may reduce *H. paradoxus* growth later in the growing season (Bush and Van Auken 1997). The different results suggest that biotic factors may also vary in their influence temporally.

Because both abiotic and biotic factors vary from year to year in both location and amount, their effects are quite often difficult to study and interpret. Specific factors may play a temporal and spatial role in *H. paradoxus* growth and distribution. Nevertheless, data from this study seem to indicate an underlying niche of abiotic constraints that need to be fulfilled and maintained at different growth stages of *H. paradoxus*.

ACKNOWLEDGMENTS

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POST-PLEISTOCENE GEOGRAPHIC VARIATION IN *JUNIPERUS COMMUNIS* IN NORTH AMERICA

Robert P. Adams
Baylor University, Gruver Lab, P.O. Box 727, Gruver, TX 79040 USA
email Robert_Adams@baylor.edu

Sanko Nguyen
Pharmacy Institute, University of Oslo, Blindern, 0316, Oslo, Norway.

ABSTRACT

Plants of *Juniperus communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, *J. c.* var. *megistocarpa* Fern. & St. John, *J. c.* var. *saxatilis* Pall. were sampled and DNA fingerprinting (RAPDs, Random Amplified Polymorphic DNAs) was performed. Based on 100 RAPD bands, *J. communis* var. *communis* and *J. c.* var. *saxatilis* from the eastern hemisphere were clearly separated from *J. communis* in North America. Populations referred to as var. *saxatilis* from the Pacific northwest did not show alliances with authentic var. *saxatilis* from Europe. However, plants with short, curved leaves, and having a white stomatal band about twice as wide as the green margins are allied with *J. c.* var. *jackii* Rehdr. Geographic variation in North American populations of *J. communis* revealed the southern Appalachian Mountains appear to have been a refugium during the late Pleistocene (Wisconsin). Plants from the isolated Mt. Charleston, Nevada were very differentiated, reflecting a long period of genetic isolation. The populations at Banff and Canmore, Alberta were somewhat intermediate between *J. c.* var. *depressa* and *J. c.* var. *jackii* from Queen Charlotte Island.

KEY WORDS: *Juniperus communis*, Cupressaceae, geographic variation, Pleistocene

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The genus *Juniperus* consists of approximately 68 species and 36 varieties (Adams, 2004). All the taxa grow in the northern hemispheres, except *J. procera* Hochst. ex Endl. which grows along the rift mountains in east Africa, thence into the southern hemisphere

(Adams, Demeke and Abulfatih 1993), and some of the Mediterranean *Juniperus* species such as *J. oxycedrus* L., *J. phoenicea* L., and *J. thurifera* L. that grow in the mountains of the northernmost part of Africa (Morocco, Algeria).

Juniperus communis is the only *Juniperus* species that occurs in both hemispheres. In North America, *J. communis* has been treated (Adams, 2004) as composed of as many as four varieties (Fig. 1). Most

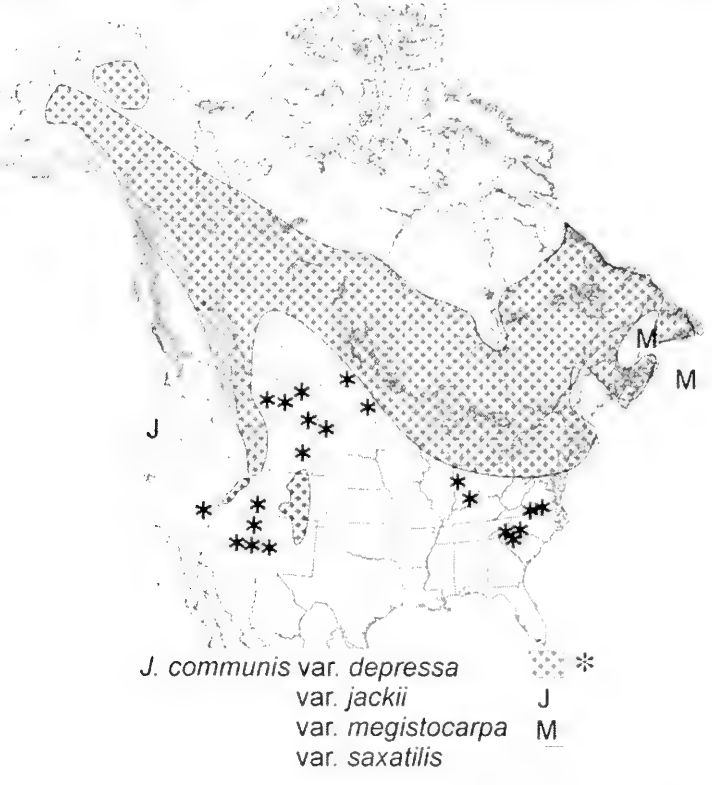

Figure 1. Distribution of *J. communis* var. *depressa*, *J. c.* var. *jackii*, *J. c.* var. *megistocarpa*, and putative *J. c.* var. *saxatilis* in North America.

of the present distribution was covered with ice during the late Pleistocene (Wisconsin), so recolonization of these areas has been recent (10-12,000 y). A number of isolated populations in the southern portion of the distributions may have served as refugia, as these areas were likely never glaciated (Fig. 1). Adams (2004) considered *J. c.* var. *jackii* to be a part of *J. c.* var. *saxatilis* in the Pacific northwest (Fig. 1).

Figure 2, from a study of Arctic populations of *J. communis* (Adams et al., 2003), revealed that these Arctic populations clustered by continent with the populations in Greenland and Iceland showing the

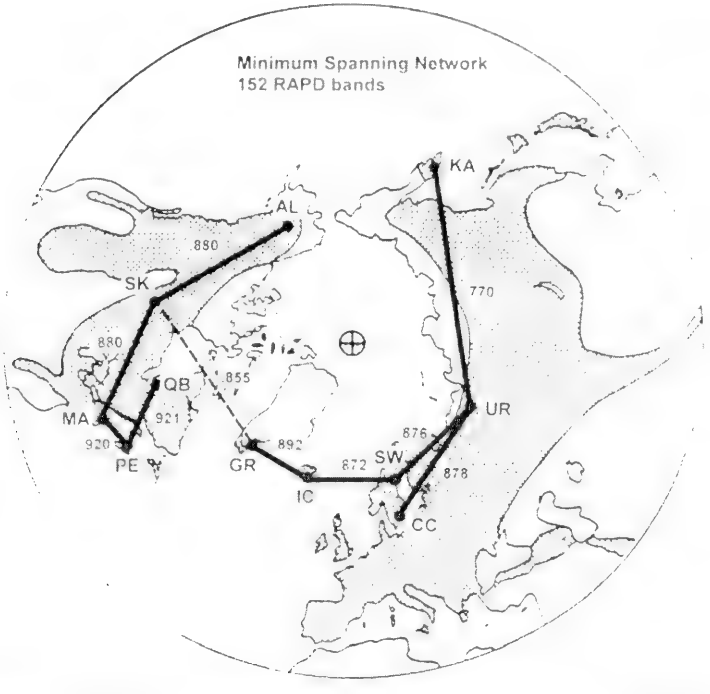

Figure 2. Minimum spanning network showing that all the North American *J. communis* populations link together and all the *J. communis* populations from the e. hemisphere link together.

highest affinities to populations from Europe, not those from North American. The North American populations were all *J. c.* var. *depressa*, whereas the eastern hemisphere populations included *J. c.* var. *communis* (CC), *J. c.* var. *saxatilis* (GR, IC, SW, UR, KA). Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland and Iceland came from Europe and not North America.

Analysis of the currently named *Juniperus communis* varieties (Adams and Pandey, 2003), resolved these taxa (Fig. 3) into six major groups: *J. communis* from Europe and central Asia (*J. communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, N. America; *J. c.* var. *saxatilis* Pall.); *J. c.* var. *megistocarpa* Fern. & St. John, Quebec; *J. c.* var. *nipponica* (Maxim.) E. H. Wilson, Japan; and putative *J. c.* var.

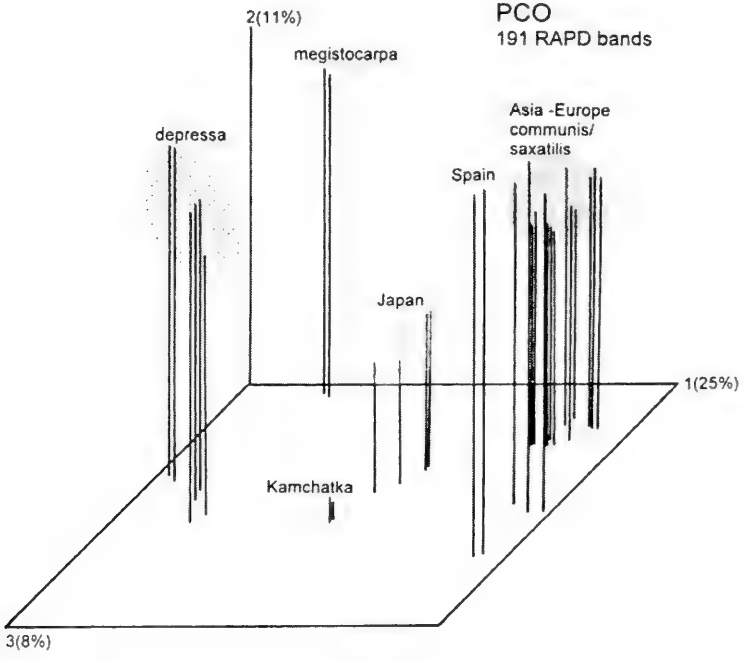

Figure 3. PCO of *J. communis* varieties (from Adams and Pandey, 2003). See text for discussion.

saxatilis, Kamchatka, Russia. However, Adams and Pandey (2003) did not include *J. c.* var. *jackii*, nor putative *J. c.* var. *saxatilis* from the Pacific northwest, USA/ Canada in their analysis.

Ashworth, et al. (1999, 2001) used DNA fingerprinting to examine *J. communis* plants identified as *J. c.* var. *depressa*, *J. c.* var. *jackii* Rehder, *J. c.* var. *montana* Aiton (= *J .c.* var. *saxatilis* Pall. see Adams, 2004) collected from California, Oregon, Nevada or Utah in the southwest and west coast of the United States. They did not get a clear pattern separating these taxa, and concluded that their samples represent a single varietal taxon. However, it not clear if they utilized population samples to remove spurious variation in RAPD bands.

In the present study, we have collected additional samples of putative *J. c.* var. *saxatilis* from the Pacific northwest, *J. c.* var. *jackii* from nw California and *J. c.* var. *depressa* from the southernmost locations in North America (Mt. Charleston, Nevada and Mt. Satula, North Carolina).

MATERIALS AND METHODS

Specimens used in this study: *J. communis* var. *communis* : Adams 7846, 7848, Stockholm, Sweden; *J. c.* var. *depressa*: Adams 7582, 7582, Denali National Park, Alaska, USA; Adams 7094, 7095, on granite bluff, Neimembian Lake, Saskatchewan, Canada; Adams 10366, 10367, Hudson Bay, Quebec, Canada (ex N. Dignard); Adams 10317, 10318, on glacial till, Canmore, Alberta, Canada; Adams 10282, 10283, Mt. Charleston, Clark Co. NV, USA; Adams 10225, 10226, on granite, Mt. Satula, Macon Co., NC, USA; *J. c.* var. *jackii*: Adams 10287, 10288, serpentine, Del Norte Co., CA, USA; *J. c.* var. *megistocarpa*, Adams 8575, 8576, Magdalene Islands, Quebec, Canada (ME); *J. c.* var. *saxatilis*: Adams 10467, 10467 (Mtns.), 10481, 10482 (coastal) Norway (ex. J. Karlsen); Putative *J. c.* var. *saxatilis*: Adams 9181, 9182 (ex. J. W. Leverenz), Esso, Kamchatka Peninsula, Russia; Adams 10304, 10305, Queen Charlotte Isl., BC; Adams 10300, 10301, on volcanic rock, Mt. Hood, Wasco Co., Oregon, USA; Adams 10328, 10329, possible hybrids, Hoodoos, Banff, BC, Canada. Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA). The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5'-3'): 134, AAC ACA CGA G; 153, GAG TCA CGA G; 184, CAA ACG GAC C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 268: AGG CCG CTT A; 338, CTG TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 375, CCG GAC ACG A; 431, CTG CGG GTC A; 478, CGA GCT GGT C.

PCR stock solutions (Taq, primer, and buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams, Flournoy and Pandey, 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM MgCl2, and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams, Flournoy and Pandey, 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating/ cooling block. The thermal cycle used was: 94° C (1.5 min) for initial strand separation, then 40 cycles of 40° C (2 min), 72° C (2 min), 91° C (1 min). Two additional steps were used: 40° C (2 min) and 72° C (5 min) for final extension. The temperature inside a PCR tube containing 15 µl buffer was monitored with a temperature probe, quantitated and printed for each step for each of the 40 cycles for every PCR run (Adams, Flournoy and Pandey, 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were

photographed over UV light using Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring. Bands were scored as present (1) and absent (0). Bands that were inconsistent in replicate analyses were not scored.

Associational measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). It should be noted that problems of homology of RAPD DNA bands on agarose gels can be significant (Rieseberg, 1996), but these errors can be accounted for using multivariate statistical methods (PCO) (see Adams and Rieseberg, 1998). A minimum spanning diagram was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in a network (Adams, et al. 2003).

RESULTS AND DISCUSSION

The major trend (figure 4) among the taxa is the separation of the eastern hemisphere plants (*J. communis* var. *communis, J. c.* var. *saxatilis,* and putative *J. c.* var. *saxatilis*, Kamchatka) from the western hemisphere plants *(J. c. var. depressa, J. c.* var. *jackii*, *J. c.* var. *megistocarpa*, and putative var. *saxatilis*). The resolution (figure 4) of *J. c.* var. *jackii* (and plants from nearby Mt. Hood) is in contrast to the report by Ashworth, et al. (1999, 2001). The Banff, Alberta individuals (putative hybrids) are intermediate between the coastal, short, curved leaved plants (Queen Charlotte Islands plants, var. *jackii*) and *J. c.* var. *depressa* (figure 4). *Juniperus c.* var. *megistocarpa* is distinct from *J. c.* var. *depressa.*

The most interesting facet of this PCO is that putative *J. c.* var. *saxatilis* (Queen Charlotte Islands, Mt. Hood, OR, and *J. c.* var. *jackii* plants) do not cluster with *J. c.* var. *saxatilis* (Norway, mountain, figure 4). It appears that the short, curved leafed taxon from the Pacific northwest thence into Alaska is part of a variable taxon, *J. c.* var. *jackii.*

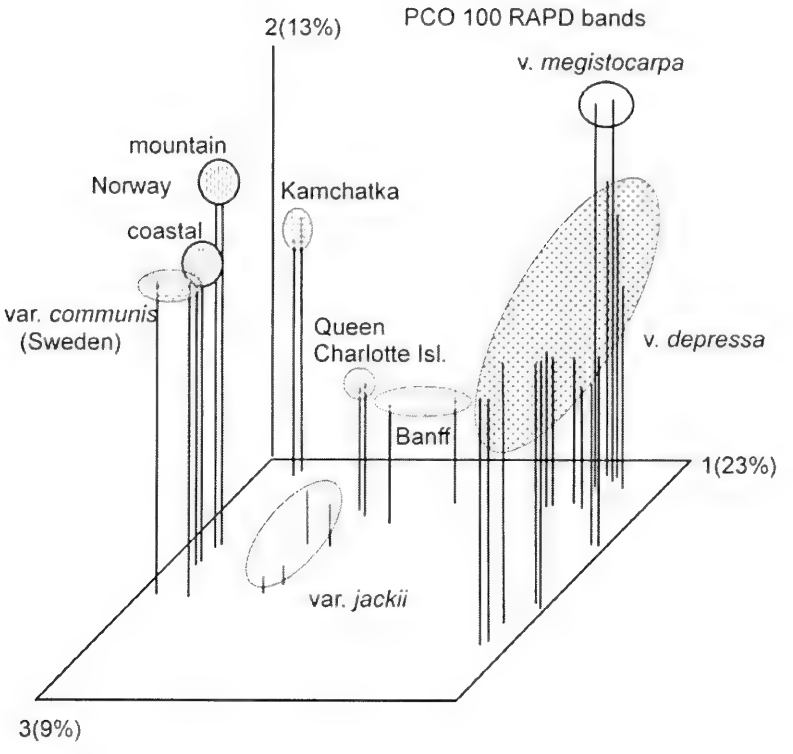

Figure 4. PCO based on 100 RAPD bands. See text for discussion.

To examine the geographic trends, the plants from the eastern hemisphere and the intermediate Banff individuals were removed from analysis and a new similarity matrix was constructed. Contouring the clustering is shown in figure 5. The major trend shows that three very geographically separated populations in Denali National Park, Alaska, (D), Hudson Bay, Quebec (H), and Mt. Satula, North Carolina (NC) are the least genetically differentiated. Certainly, there are numerous bridging populations between Alaska and Hudson Bay, but the North Carolina population is quite distant from adjacent populations.

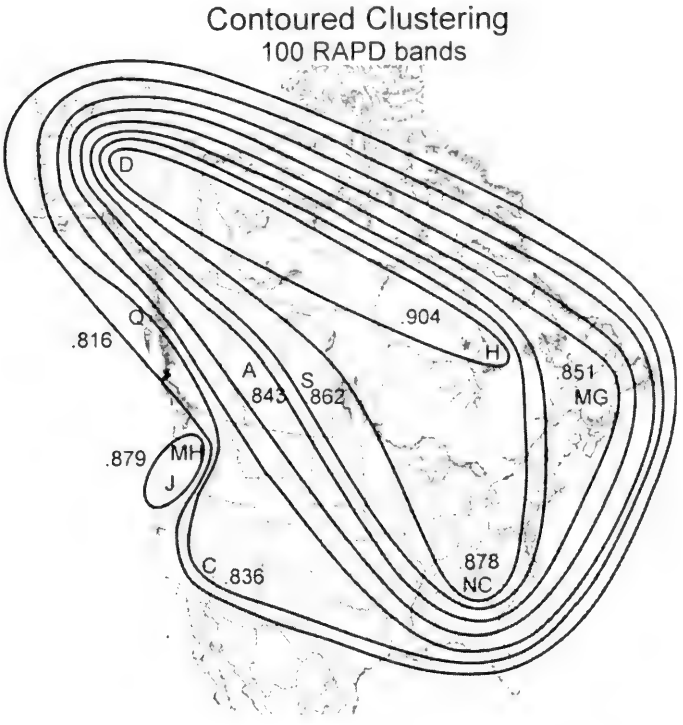

Figure 5. Contoured clustering based on 100 RAPD bands using minimum spanning values.

The second facet of the contoured clustering (figure 5) is that *J. c.* var. *jackii* and Mt. Hood, OR plants are divergent from the bulk of the North America *J. c.* var. *depressa* populations. The Queen Charlotte Islands (Q) are also quite divergent, but fail to link with the *J. c.* var. *jackii* group. It seems likely that all these infra-specific populations are interfertile and that introgression from *J. c.* var. *depressa* may be occurring into the Queen Charlotte Island plants. The Queen Charlotte Islands plants do, however, maintain short, curved leaves like *J. c.* var. *jackii*.

Another interesting trend is that J. c. var. *megistocarpa* (large fruited, common juniper on sand dunes, MG) is more similar (figure 5)

to most *J. c.* var. *depressa* populations than the Mt. Charleston (C) population is to *J. c.* var. *depressa.*

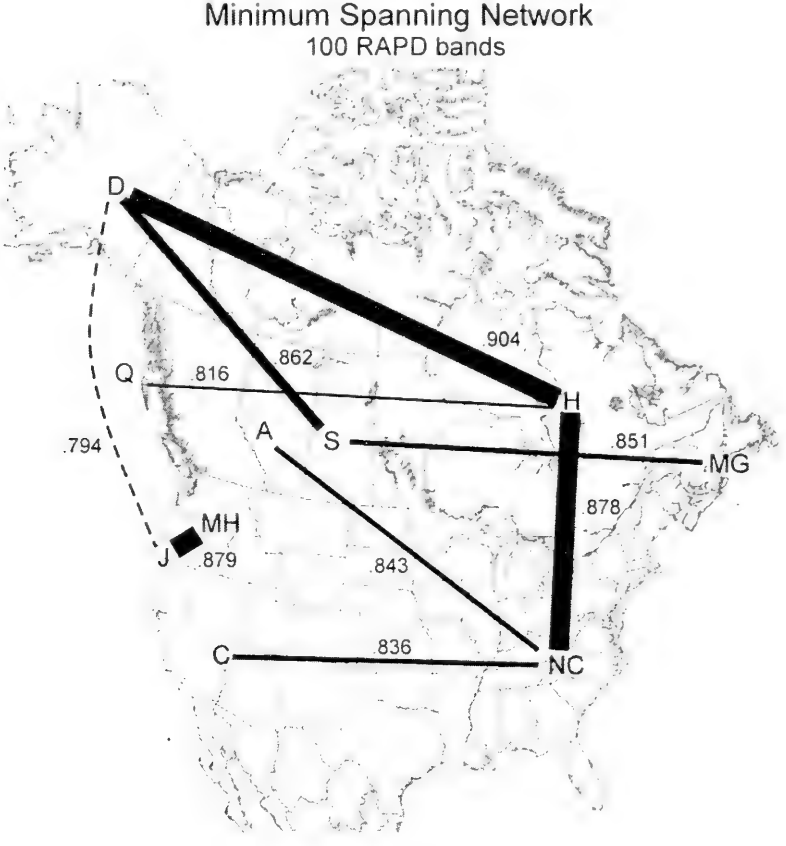

Figure 6. Minimum spanning network based on 100 RAPD bands. The line width is indicative of the similarity between populations.

Examination of the linkage among populations shows (figure 6) a strong north-south and southeast-northwest linkage. *Juniperus c.* var. *megistocarpa* (MG) links with *J. c.* var. *depressa* from Saskatchewan (S). The adjacent population of *J. c.* var. *depressa* in Alberta (A) links with the North Carolina (NC) population. The Queen

Charlotte Islands population (Q) links with the Hudson Bay (H) plants, which also have short, curved leaves. The leaf length and curvature may be somewhat environmentally induced in the colder locations. The *J. c.* var. *jackii* populations (J, MH) link at a lower level to the Denali, Alaska (A) plants.

It is possible that the Alaska (A) population was not glaciated during the Wisconsin (figure 7), but all the other northern populations were glaciated. Only the Alaska (D), Mt. Charleston (C) and North

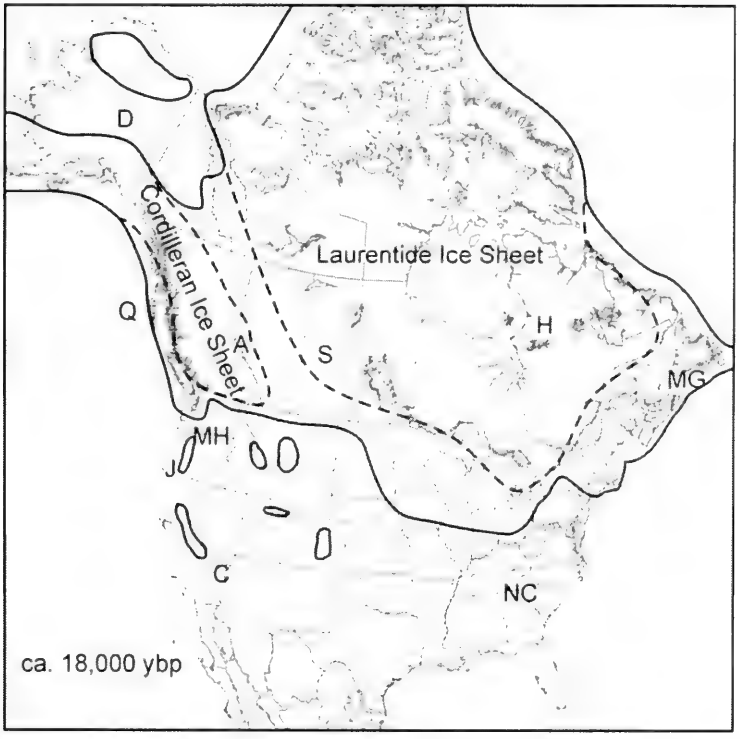

Figure 7. Maximal ice cover during the late Wisconsin. Notice that only the Alaska (D), Mt. Charleston (C) and North Carolina (NC) populations may have survived *in situ* or nearby.

Carolina (NC) populations may have survived during the Wisconsin. The *J. c.* var. *jackii* populations (J, MH) likely moved to lower elevations. However, the northwestern California population of *J. c.* var. *jackii* presently occurs on serpentine, so it seems unlikely that this edaphic type grew on serpentine at a lower elevation. *Juniperus* is well known to be very adaptive to edaphic conditions, so Wisconsin era genotypes may have merely invaded the largely open habitat on the serpentine of northwestern California and southwestern Oregon.

There seem to be four possible refugia during the Wisconsin: southern Appalachian Mts. (cf. NC); southern Rocky Mountains (cf. Mt. Charleston and Arizona/ New Mexico Mts.; central Sierra Nevada; and possibly an ice free corridor in central Alaska. It is easy to imagine that birds carried seeds from plants from the southern Appalachians northward into northern US and Canada. It appears more likely that the southern Appalachians were the source of germplasm in re-colonization of Canada than the southern Rocky Mountains. Notice, figure 6, that the linkage of Mt. Charleston (C) is to North Carolina (NC) rather than to Alberta (A) or Saskatchewan (S). The relictual status of the Alaska (D) population is uncertain. It does show strong linkage with both Hudson Bay and Saskatchewan populations (fig. 6) and it could have been the source of germplasm in recolonization of Canada. Conversely, the Alaskan population may have not survived the Wisconsin and it may have been re-colonized with seeds from central Canada.

The *J. c.* var. *jackii* group was distinct in this analysis; however, the addition of samples from central California near Mono Lake, CA, the Puget Sound/ Vancouver area, Idaho, and Alaska may change our concepts of the short, curved leafed *J. communis* group.

At present, it seems prudent to recognize three varieties of *J. communis* in North America: var. *depressa* with long, straight leaves, stomatal band width 1 to 1.5 x width of green side band (figure 8); var. *jackii* with short, curved leaves, stomatal band 2 x width of green side band from central California to Alaska (figure 8);and var. *megistocarpa*, with large female cones, restricted to sand dunes and rocky beaches in far ne Canada (figure 8).

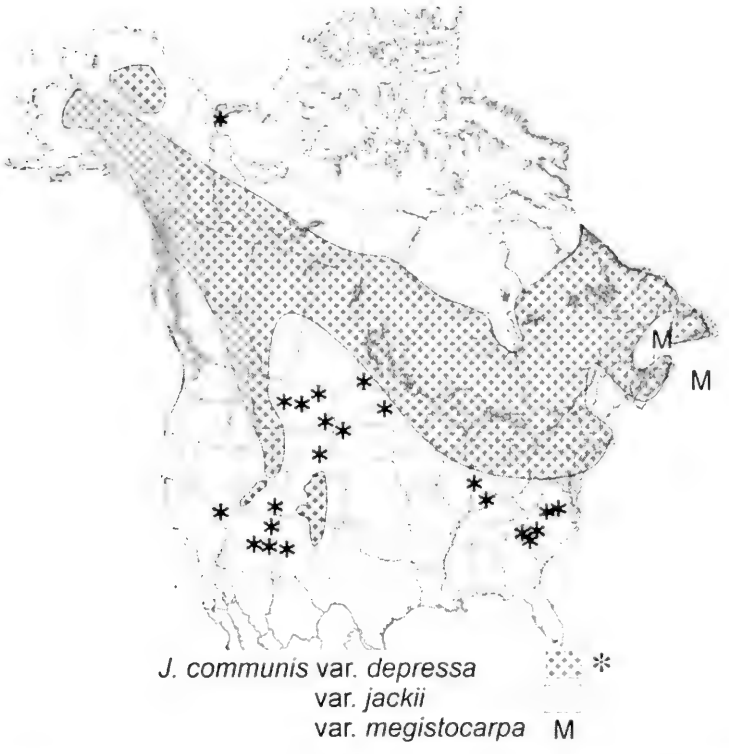

Figure 8. Distribution of *J. communis* in North America. The range of the *J. c.* var. *jackii* genotype has not been verified except in nw California and w Oregon.

This analysis indicates that *J. c.* var. *saxatilis*, as known in Europe and the eastern hemisphere, is not represented in North America. It is not clear from this study if *J. c.* var. *jackii* is confined to n California and Oregon or is widespread from California to Alaska. The distribution of *J. c.* var. *jackii* (figure 8) is based on leaf morphology suggesting that a single variety exists in the northwestern US, western Canada and Alaska. Additional sampling and DNA analysis is needed to resolve this problem.

ACKNOWLEDGEMENTS

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KEYS TO THE FLORA OF FLORIDA: 15, *TYPHA* (TYPHACEAE)

Daniel B. Ward
Department of Botany, University of Florida
Gainesville, Florida 32611, U.S.A.

ABSTRACT

Typha (Typhaceae) is represented in Florida by 3 species and 1 hybrid. *Typha latifolia* and *T. domingensis* are widespread and common. *Typha angustifolia* is very infrequent and is believed to be of recent introduction. *Typha glauca*, the hybrid of *T. latifolia* and *T. angustifolia*, is present but is excluded from the flora in belief that its colonies do not persist. Putative hybrids between *T. latifolia* and *T. domingensis* have been reported but were not observed. An amplified key is given to the Florida taxa.

KEY WORDS: *Typha*, Typhaceae, Florida flora.

The cat-tails (*Typha*, Typhaceae) are among the easiest of plants to identify correctly to genus. To identify *Typha* correctly to *species* is another story.

An excellent, compendious treatment of *Typha* in the Southeast has been prepared by John W. Thieret and James O. Luken (Harvard Papers in Botany 1:27-56. 1996). Highly competent treatments (though they differ in some interesting ways) of the North American species have been presented by Neil Hotchkiss and Herbert L. Dozier (Amer. Midland Nat. 41:237-254. 1949) and by S. Galen Smith (Fl. N. Amer. 22:278-285. 2000). Hotchkiss had the opportunity to collect and study cat-tails to the southernmost limit of their Florida range, and is the source of much original data. Smith, though limited in examination of the more southern populations, had the advantage of greater specimen access and incorporation of recent observations by other authors. His earlier work (Smith, Amer. Midland Nat. 78:257-287. 1967) is the basis

for present understanding of hybrids among the North American species.

It is agreed that in eastern North America there are three species and one common hybrid of *Typha*. The most widespread of these, and formerly the most abundant, is *T. latifolia* L., the Common Cat-tail. Once restricted to eastern provinces and now increasingly far-ranging is *T. angustifolia* L., the Narrowleaf Cat-tail. Mostly limited to coastal areas but frequent in peninsular Florida is *T. domingensis* Pers., the Southern Cat-tail. A fourth entity, *T. glauca* Godron, the hybrid of *T. angustifolia* and *T. latifolia*, is often locally abundant, even dominant, wherever its two parents occur together.

It is this hybrid that causes most taxonomic uncertainty and misidentifications. Within Florida, the hybrid was first observed and collected in 1948 along a tributary of the St. Johns River, Duval County (Hotchkiss 7266 - FLAS). It was found again at the same location in 1963 (Ward 3505 - FLAS) and has been intermittently monitored to the present.

Though hybrids of *Typha domingensis* and *T. latifolia* are known elsewhere (Smith, 1967), and have been reported for Florida (Smith, 2000), they seem wholly unknown by Florida botanists (E. West, R. K. Godfrey, pers. comm., ca. 1965; D. W. Hall, July 2005). Specimens annotated as "most probably" *T. domingensis* - *T. latifolia* hybrids (G. Smith, 1984 - FLAS) were recognized in the field by their collectors either as *T. angustifolia* (Ward 3508 - FLAS) or as *T. glauca* (Hotchkiss 7266, Ward 3505 - FLAS).

The characters used in keying specimens of the three species and their hybrid are, in part, difficult to see in the laboratory and of little utility in the field. The stigmas of fertile flowers may be linear (as in *T. domingensis* and *T. angustifolia*), or lance-ovate, broader than the styles (much broader in *T. latifolia*, less so but clearly widened in *T. glauca*). Care must be taken it is the stigmas that are observed, and not the numerous intermixed clavate-tipped sterile flowers. Observation is further impeded by the innumerable long hairs borne on the pedicels of

both fertile and sterile flowers. The pollen of *T. latifolia*, the grains cohering in tetrads, is unique in North American species, but requires microscopic examination.

A more satisfying, readily observed character is the absence of a gap between the pistillate and staminate spikes of *Typha latifolia*. This trait, confirmed by the clearly broadened stigmas, is usually sufficient for confident identification of the Common Cat-tail.

Typha domingensis and *T. angustifolia* invariably have a prominent gap, usually of more than 1 cm., between the pistillate and the staminate portions of their inflorescence; this trait remains still visible as a length of smooth shaft even after the male flowers have fallen, leaving their rough bases on the upper axis. *Typha glauca* also characteristically shows this pistillate-staminate gap, though it is sometimes quite small, at times no more than a few millimeters n width.

The overall dimensions of the mature pistillate spikes, and particularly their length/width ratios, is of high diagnostic value. *Typha latifolia* pistillate spikes are chubby, above 1.5 cm. in thickness, with a l/w ratio of 4 to 5. *Typha angustifolia* pistillate spikes are strikingly slender, usually less than 1 cm. thick, with a ratio of 20 to 25. *Typha domingensis* and *T. glauca* are intermediate both in thickness and in l/w ratio. Immature inflorescences are more slender, thus deceptively imply a larger ratio than will be present with maturity. Young inflorescences of *T. domingensis* are especially subject to misidentification as *T. angustifolia*, an error that can be avoided by noting the lighter color and usually greater length of *T. domingensis* spikes.

In addition to the differences employed in the accompanying key, the taxa of *Typha* are distinguishable in the field by gross characteristics that do not survive transfer to the herbarium. *Typha domingensis* is a tall plant, regularly above one's head and frequently to 3 m. or more. Its mature pistillate spikes are a rich cinnamon brown, a hue not found in any of the other taxa. *Typha latifolia* is of medium

height as compared with the other species. Its leaves are medium green, and its pistillate spikes are dark "chocolate" to blackish brown. *Typha angustifolia* is by far the smallest, seldom reaching above 1.5 m. Its leaves have a blue-green cast, while its pistillate spikes are dark brown, identical in color with those of *T. latifolia*. *Typha glauca* is intermediate to the characteristics of *T. angustifolia* and *T. latifolia*. Its leaves are dark green, and its pistillate spikes are similar in their dark brown color to that of its parents.

The present distribution of *Typha angustifolia*, throughout North America and into Florida, is not fully understood; nor is its nativity established. Though its distribution has been mapped (Hotchkiss & Dozier, 1949), its range is continuing to expand, as is that of the hybrid, *T. glauca* (Smith, 2000). Recent observation in northern Indiana, across New York state and into Ontario (D.B.W. obs., 2002, 2005), found *T. glauca* to be the common form. Only in northern Ontario (Bruce Peninsula) was *T. latifolia* present in any numbers. The Montezuma Marshes of upstate New York, emphasized by Hotchkiss and Dozier (1949) as holding *T. latifolia*, *T. angustifolia* and their hybrid, now appears to be a uniform stand of *T. glauca*. Other than a brief abstract characterizing *T. angustifolia* as a "foreigner" (R. L. Stuckey & D. P. Salamon, Ohio J. Sci. 87 (abs.):4. 1987) and references to that note, there is no published expression of a likely non-native status of this species. In Florida, though not always seen in former years, *T. angustifolia* is marginally present at its original station in Duval County. *Typha glauca* appears no longer present, and there is little evidence of either taxon's presence elsewhere. Whatever the status of the species to the north, the absence of *Typha angustifolia* from Georgia (Hotchkiss & Dozier, 1949; S. B. Jones & N. C. Coile, Distr. Vasc. Flora of Georgia, 1988) and its disjunct presence in Florida only in a single long-disturbed coastal marsh justifies its treatment here as an introduction.

The observations of this study, though limited, suggest that when *Typha angustifolia* arrives at a site and comes in contact with *T. latifolia*, hybridization quickly follows. The hybrid, *T. glauca*, expands vigorously by vegetative reproduction, out-competing and soon

displacing the native species. With adverse conditions, possibly drought or high water, the plants are adversely impacted (S. G. Smith, Arch. Hydrobiol. 27:129-138. 1987), with the genus perhaps even locally eliminated. But when conditions again become suitable, *T. latifolia* from nearby sources re-enters the site, while *T. glauca* remains absent until fertile *T. angustifolia* can be re-introduced from farther-away sources, forming the hybrid anew, and repeating the cycle.

The name "*Typha angustifolia*" as used in Florida far antedates the 1948 discovery of the species in Duval County. Early misapplication of this name to the native *T. domingensis* has filled the nation's herbaria and older literature (e.g., J. K. Small, Man. S.E. Flora, 1933) with reports of its seemingly widespread distribution. Only with the work of Hotchkiss and Dalziel (1949) was the error noted and corrected.

The word "Typha" is of ancient usage, apparently consistently applying to the genus as presently known. That the name may have come from the Greek, *typhein*, has been suggested. But the ascribed meaning, "to smoke or emit dense smoke," probably suffers from a too-rigid translation. The proposed "allusion either to the use of these plants for maintaining smoky fires or to the smoky-brown color of the fruiting spikes" (Thieret & Luken, 1996) is conjectural and surely incorrect. Perhaps the translation may also be read as "cloud" and is in reference to the pollen itself, released in profuse quantities, sufficient to be gathered and once employed as a food (D. F. Austin, Florida Ethnobotany, 2004). Still more likely is the simple derivation of the Greek, *typhos*, "of marshes."

Though in recent years the name *Typha domingensis* has uniformly been used for the Southern Cat-tail, a shadow has overlain the correctness of its epithet. This cat-tail was first named by C. H. Persoon (Synopsis Plantarum 2:532. 1807), but at an ambiguous rank. In his listing of the plants of the West Indies and their descriptions, Persoon routinely numbered his species. But under *Typha*, after "*latifolia*" which he numbered "1", and before "*media*" which he numbered "2", Persoon inserted his new "*domingensis*" marked only by a marginal asterisk. Since Persoon inserted undoubted varieties in an

identical fashion (though marked with Greek letters), the rank he intended by an asterisk was unclear. The monographer K. Graebner (Das Pflanzenreich IV. 8:14. 1900), though he recognized *T. domingensis* at specific level, indicated that Persoon had treated it as a "subsp." of *T. latifolia*. M. L. Fernald (Rhodora 37:385. 1935) went further, acknowledging Persoon's epithet only as a trinomial, and employed *T. truxillensis* HBK. (1815) for the species. Hotchkiss and Dozier (1949) returned to *T. domingensis*, citing a footnote reference to Persoon's introduction. There, Persoon, stating "speciebus obscuris" are indicated by an "asteriscum" placed alongside, confirms his intention of specific rank.

The accompanying amplified key omits *Typha glauca* in the belief that, if included, its present rarity (or absence) in Florida and its intermediate morphology will cause an unacceptable number of misidentifications. The descriptive comments above are judged less susceptible to error. However, if such a key is desired, Smith (2000) is offered.

I am grateful to my friends Erdman West and Robert K. Godfrey, years ago, regarding their knowledge of cat-tails, to his wife, Suzanne, for her patient help in obtaining the 1963 collections, and to Robert W. Simons for his assistance in recent observations.

TYPHA L. Cat-tails [i]

1. Staminate and pistillate parts of spike contiguous, usually wholly concealing axis of spike; pistillate portion of spike dark brown at maturity, 2-3 cm. thick, relatively short and thick (length 5.5-6.0 times width); leaves 8-16 mm. broad, without visible mucilage glands on inside of upper sheath; stigmas of fertile flowers lance-ovate (intermixed with clavate-tipped sterile flowers); pollen grains cohering in 4s. Emergent aquatic perennial herb. Freshwater marshes, stream bottoms, lake shores. Throughout; common, locally forming dense monospecific stands. Spring-summer.
   COMMON CAT-TAIL. **Typha latifolia** L.

1. Staminate and pistillate portions of spike separated, showing 1-4 cm. of axis of spike; pistillate portion of spike light to dark brown, 1.3-2.2 cm. thick, relatively long and slender (length 6-10 times width); leaves 5-12 mm. broad, with small brownish longitudinally-oriented mucilage glands on inside surface of upper sheath; stigmas of fertile flowers linear (intermixed with clavate-tipped sterile flowers); pollen grains not cohering.

2. Pistillate portion of spike light brown, 15-25 cm. long, 1.5-2.5 cm. thick; leaves 8-12 mm. broad; plants tall, often to 3 m. Emergent aquatic perennial herb. Brackish marshes, less often in freshwater. Throughout peninsula, extending west along panhandle shores (to Gulf County); common in coastal areas, less so inland, absent in western panhandle. Spring-summer. [*Typha angustifolia*, misapplied; *Typha truxillensis* HBK.]
SOUTHERN CAT-TAIL. **Typha domingensis** Pers.

2. Pistillate portion of spike dark brown, 8-18 cm. long, 1.2-1.8 cm. thick; leaves 5-10 mm. broad; plants relatively small, rarely above 1.5 m. Emergent aquatic perennial herb. Brackish or freshwater marshes. Northeast coastline (Duval, St Johns counties); rare. Spring. Plants of *T. domingensis* with immature spikes are commonly identified as this. An aggressive invader in northeast and midwestern states where now displacing the native *T. latifolia*, but thus far showing no such behavior in Florida.
NARROWLEAF CAT-TAIL. * **Typha angustifolia** L.

Excluded names: **Typha glauca** Godron

A hybrid between *T. angustifolia* and *T. latifolia* (Hotchkiss & Dozier, 1949), and known in Florida only from Duval Co. where these species occur together. Plants are essentially sterile and do not appear to form self-sustaining populations.

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i. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. This paper is a continuation of a series begun in the 1970s (*vide* Phytologia 35:404-413. 1977). Keys are being prepared for all genera of the Florida vascular flora, but the present "amplified" series is restricted to genera where a new combination is required or a special situation merits extended discussion.

ERIOGONUM GRANITICUM (POLYGONACEAE), A NEW NAME AND RANK FOR *E. TENELLUM* VAR. *RAMOSISSIMUM*

B. L. Turner
Plant Resources Center
The University of Texas at Austin
Austin, Texas 78712
email billie@uts.cc.utexas.edu

Eriogonum graniticum B.L. Turner, **nom. & stat. nov.**

Based upon *Eriogonum tenellum* var. *ramosissimum* Benth., in DC., Prodr. 14: 20. 1856. - not *E. ramosissimum* Eastwood 1896.

In Bentham's protologue two specimens were cited, one by Lindheimer (no. 683, which was collected, September of 1847, N of Fredericksburg in "Granitic mountains," according to Blankenship [1907]") and another by Riddell (no. 23). Reveal, by annotation (GH) selected the former sheet as lectotype, although strict adherence to the Code would mandate the selection of one of the two sheets examined by Bentham himself, these housed at KEW. Regardless, I have opted to elevate Bentham's var. *ramosissimum* to specific rank, as noted in the account that follows.

In the Atlas of Texas Plants (Turner et al. 2002) I treated *E. tenellum* var. *ramosissimum* as a species, dubbing this "*E. ramosissmum*" in my map of the taxon. Fortunately, I did not make the name concerned formal since, as indicated in the above, that name is preoccupied at the specific level. The present note is designed to correct this over sight.

Reveal (1968, 1970, 1976) treated *Eriogonum* for Texas and adjoining areas. He recognized *E. tenellum* as having three varieties: var. *tenellum* of far western Texas, New Mexico, and Arizona, this having strictly basal leaves; var. *platyphyllum* with cauline leaves, this largely confined to calcareous soils of southern Trans-Pecos, Texas and adjacent Mexico; and var. *ramosissimum,* also with cauline leaves, this confined to granitic out crops in central Texas. Reveal (2005)

maintained this triad, each at the varietal level, in his recent account for the Flora of North America. Nevertheless, I treated all of these at the specific level in the above mentioned Atlas of Texas Plants. As well noted by Reveal (1968), the var. *ramosissimum* is easily and consistently recognized by its "elliptic to deltoid leaves sheathing up the stems to 15 cm" while leaves of the var. *platyphyllum* "are ovate to orbicular and sheath up the stems to 20 cm."

In short, the three varietal taxa of *E. tenellum* appear to be good biological species, each existing in organized populations that show little sign of intergradation, although the occasional hybrid or its derivitive can be expected between *E. tenellum* and *E. platyphyllum* where they grow together. On the other hand, *E. graniticum,* in that its distribution is confined to granitic soils in the Central Mineral Region of Texas, is not likely to hybridize with yet other taxa.

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MULGEDIUM OBLONGIFOLIUM (ASTERACEAE), A NEW COMBINATION

James L. Reveal

University of Maryland, College Park, MD 20742-5815
New York Botanical Garden, Bronx, NY 10458-5126
Mailing Address: 18625 Spring Canyon Road, Montrose, CO 81401-7906, U.S.A., jreveal@umd.edu

ABSTRACT

Mulgedium oblongifolium (Nutt.) Reveal is the correct name for *M. pulchellum* (Pursh) G. Don because *Lactuca oblongifolia* Nutt. (ante Aug 1813) predates *Sonchus pulchellus* Pursh (Dec 1813) as pointed out by Reveal in 1968.

KEY WORDS: *Lactuca*, *Mulgedium*, *Asteraceae*, Flora of North America

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In *Flora of North America North of Mexico*, Strother (2006) stated that if the diagnostic phrases used by Nuttall in Fraser Brothers's 1813 catalog (reprinted by Greene 1890) were adequate, then *Mulgedium pulchellum* (Pursh) G. Don (in R. Sweet, Hort. Brit., ed. 3: 418. 1839) would be a later name. Inasmuch as, in 1813, no perennial blue flowered species of *Lactuca* were known from the New World, the use of these two diagnostic features constitutes a valid diagnosis (Reveal 1968). Nuttall is now considered to be the author of the names in the catalog (Art. 46, Ex. 3; McNeill et al. 2006, contrary to Shinners 1956) and the names are valid (Cronquist et al. 1956; Reveal 1968, contrary to Shinners 1955). The concept of a "nomen subnudum" that once prevailed informally in the systematic community was never permitted by the *International Code of Botanical Nomenclature* and is not allowed in the present *Code* (McNeill et al. 2006), and while *Lactuca oblongifolia* Nutt. was rejected by Cronquist et al. (1956) for this reason, their rationale has no basis as the name does have a validating description. Furthermore, Nuttall's names in the Catalogue were accepted by the author as there was no statement by Nuttall that he was not accepting the names (Art. 34.1(a); Reveal 1968). Because

Sonchus pulchellus Pursh (Fl. Amer. Sept. 2: 502. Dec 1813) is a later synonym (but based on the same Nuttall collection gathered along the Missouri River in 1811 and used by Nuttall to establish *L. oblongifolia*) the following combination is proposed:

Mulgedium oblongifolium (Nutt.) Reveal, **comb. nov**. Based on *Lactuca oblongifolia* Nutt., Cat. Pl. Upper Louisiana: unpaged [no. 47]. ante Aug 1813.

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A NEW SPECIES OF *ORBEXILUM* (LEGUMINOSAE) FROM CHIAPAS, MEXICO

B. L. Turner
Plant Resources Center
The University of Texas at Austin
Austin, Texas 78712
email billie@uts.cc.utexas.edu

ABSTRACT

In his treatment of the tribe Psoraleeae for the New World, Grimes (1990) recognized 12 species of the genus *Orbexilum,* all of these confined to North America. At least one of these, *O. melanocarpum,* was broadly conceived, containing elements that are herein considered worthy of formal recognition, namely ***O. oliganthum*** (Brandegee) B.L. Turner, **comb. nov.** of northcentral Mexico, and ***O. chiapasanum*** B.L. Turner, **sp. nov.,** of Chiapas, Mexico. A discussion of their relationships is provided, along with maps showing their distributions.

KEY WORDS: Leguminosae, *Orbexilum*, Mexico

Grimes (1990) provided a systematic account of the genus *Orbexilum.* In this he recognized *O. melanocarpum* (Benth.) Rydberg as a widespread variable species of Mexico encompassing both *Psoralea oliganthum* Brandegee, and the presently described novelty, *O. chiapasanum.*

ORBEXILUM CHIAPASANUM B.L. Turner, **sp. nov.** Fig. 1

Orbexilo melanocarpo (Benth.) Rydb. similis sed differt plantis altioribus, leguminibus brevioribus (ca. 6 mm vs. 10 mm) in secco magis penbitus nigris et seminibus minoribus (ca. 3 mm vs. 6 mm).

Perennial, rhizomatous, sprawling herbs to 2 m high. **Primary stems** erect, ciliate, upwardly appressed-pubescent. **Mid-stem leaves** trifoliate; petioles 2-4 cm long; terminal leaflets ovate, 3-4 cm long, 1.5-2.5 cm wide. **Racemes** 10-15 cm long, the peduncles mostly 6-10

cm long. **Calyx** 6-7 mm long, glandular-punctate, the lobes 4-5 mm long. **Flowers** purple to violet-purple; petals 7-8 mm long, the banner purple with a white eye. **Legumes** ovoid, ca. 6 mm long, 4 mm wide; seeds ca. 3.5 mm long, 2.0 mm wide.

TYPE: **MEXICO. CHIAPAS:** Mpio. Amatenango del Valle, 1835 m, 12 Jun 1945, *E. Matuda 5821* (Holotype: LL; isotype TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. CHIAPAS: Mpio. La Trinitaria.** E of Laguna Tziscao, Monte Bello Natl. Park, 1300 m, 18 Nov. 1980, *Breedlove & Almeda 47547* (LL). **Mpio. Ocosingo**. 21.2 mi from the intersection with hiway 190, on road to Palenque, 11 Mar 1985, *Grimes 2620, 2628* (TEX). Mpio. Oxchuc: 5 mi ESE of Oxchuc, 6 Mar 1985, *Grimes 2619* (TEX). San Juan Cancuc, Ohteel, 5000 ft, 21 Apr 1992, *Brett 931* (TEX).

As indicated in Fig.1, *O. chiapasanum* is confined to the state of Chiapas, Mexico, hence its name. It is readily distinguished from both *O. melanocarpum* and *O. oliganthum* by its taller habit and smaller legumes having smaller seeds.

Orbexilum oliganthum (Brandegee) B.L. Turner, **comb. nov.** Fig. 2
Based upon *Psoralea oligantha* Brandegee, Univ. Calif. Public. Bot. 4: 179. 1911.

The type of this taxon is from the higher elevations of Sierra de Parras, southern Coahuila, Mexico. Grimes (1990), in his reduction of this taxon to synonymy under his broad concept of *O. melanocarpum*, notes:

"Some populations found above 2400 m on mountain ranges in Nuevo Leon, Coahuila and Zacatecas consist of diminutive plants usually 15 cm tall or shorter, with leaflets 3.0 cm long or less, flowers 9-10 mm long, broadly acute calyx teeth, and fruits with beak about 2.5 mm. These plants correspond to *Psoralea oligantha* Brandegee. However, the character states are not unique to these mountain populations, and are not found on all populations within the same area. The broadly acute calyx teeth, perhaps the character most constant in these populations, grade into the more typical linear-triangular teeth characteristic of the species."

I cannot agree with the submergence of this taxon within *O. melanocarpum*. There appears to be a syndrome of characters which mark the species, most of which are called to the fore by Grimes. Nor do the characters concerned appear to intergrade into those of *O. melanocarpum*. Combined with its high elevational habitats, and relatively restricted distribution (Fig. 2), *O. oliganthum* appears to be worthy of recognition at the specific level.

ACKNOWLEDGEMENTS

Dot maps in the present paper are based upon specimens housed at LL, TEX. I am grateful to my colleague, Guy Nesom , for the Latin diagnosis.

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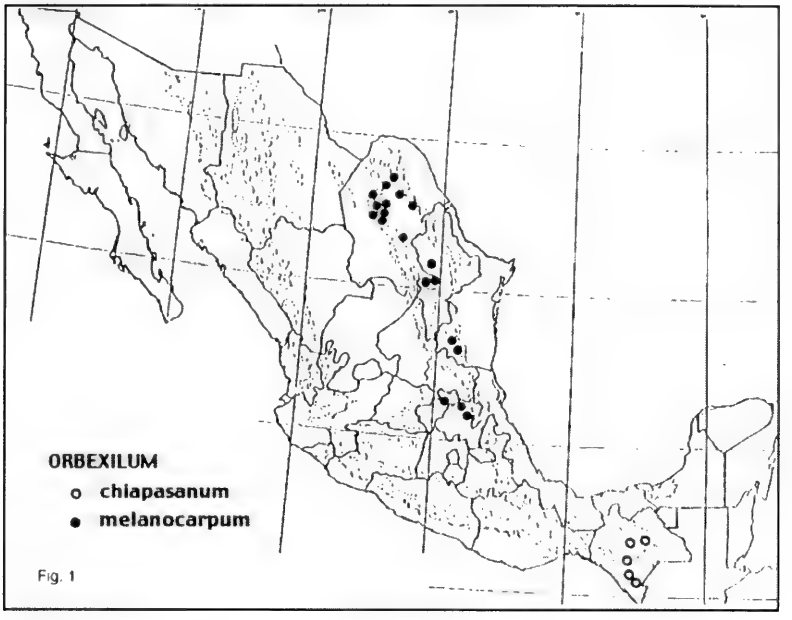

Fig. 1. Distribution of *Orbexilum melanocarpum* (closed circles) and *O. chiapasanum* (open circles).

Fig. 2. Distribution of *Orbexilum oliganthum*.

A NEW SPECIES OF *PHASARIA* (BRASSICACEAE) FROM NORTHCENTRAL MEXICO

B. L. Turner
Plant Resources Center
The University of Texas at Austin
Austin, Texas 78712
email billie@uts.cc.utexas.edu

ABSTRACT

A new species, ***Phasaria vigana*** B. L. Turner, is described from the state of Coahuila, Mexico. It is known only from limestone cliffs in the Sierra Vigas at altitudes of 11, 000-11, 600 ft. and appears to be most closely related to *P. mexicana*, although it differs markedly in habit, and characters of the inflorescence. A photograph of the type is provided.

KEY WORDS: Brassicaceae, *Physaria*, Mexico, Coahuila

Routine identification of Mexican plants has revealed the following novelty:

Phasaria vigana B. L. Turner, **sp. nov.** Fig. 1

Physariae mexicanae (Rollins) O'Kane & Al-Shehb. similes sed differt habitu acaulescenti, radicibus palaribus ligneis incrassitis, et petalis luteis (?) ac majoribus (9-10 mm longis vs. 5-8 mm).

Caespitose thick-stemmed perennials 2-3 cm high. Leaves linear-lanceolate, silvery pubescent, 1.5-2.5 cm long, 1.0-1.5 cm wide; trichomes sessile, peltate, ca. 0.25 mm across, the rays completely fused, or nearly so. **Sepals** 4-5 mm long, ca. 2 mm wide, pubescent like the leaves. Pedicels 8-10 mm long. **Petals** broadly obovate, seemingly yellow, 9-10 mm long, ca. 5 mm wide, the claws inconspicuous (ca. 1 mm long). **Single stamens** ca. 5 mm long, thickened and somewhat dilated at the base; paired stamens ca. 6 mm

long, otherwise like the singles; glandular tissue weakly developed, if at all. **Capsule** ovoid, ca. 2 mm high, 1.5 mm wide, glabrous; ovules 4 per carpel.

TYPE: **MEXICO. COAHUILA:** Sierra de Viga, ca. "29 (air) miles E of Saltillo, on the SE slopes of the Mt., ca. 6 miles E of Jame...near summit of Cerro San Rafael, on limestone cliff faces." (ca. 25 21N, 100 32 W), 11,000-11,600 ft., 15 May 1977, *James Henrickson 16135* (Holotype: TEX).

In the treatment of Rollins and Shaw (1973), and Rollins (1993), this novelty, because of its peltate vestiture, will key to or near *Physaria mexicana*. In addition to its distribution (Fig. 2), the latter differs from *P. vigana* in a number of characters, as indicated in the above diagnosis. Indeed, it is perhaps likely that *P. vigana* is a localized subalpine endemic that has evolved out of *P. mexicana*. Rollins and Shaw (1973) note that the latter taxon has white or lavender petals, and that it occurs in the Sierra Azul, Sierra Encantada, Sierra de la Madera, Sierra Negra and Sierra Parras at elevations of "5, 000 to 7, 500 feet." This stands in marked contrast with the altitude given on the type of *P. vegana*. Whether or not the latter has yellow petals (when fresh), remains to be seen; however, the dried petals, to my eye at least, appear to be yellow.

The species is named for the Sierra in which it was first collected.

ACKNOWLEDGEMENTS

I am grateful to my colleague, Guy Nesom, for reviewing the paper and providing the Latin diagnosis.

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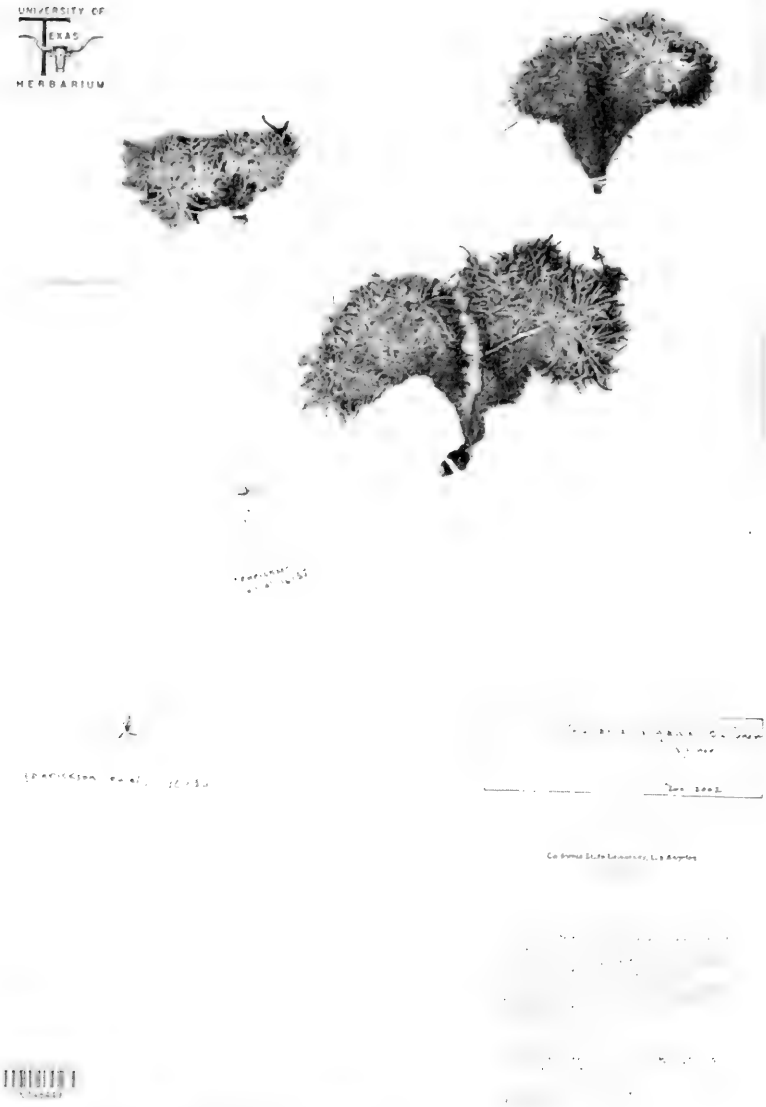

Fig. 1 Holotype of *Phasaria vigana*.

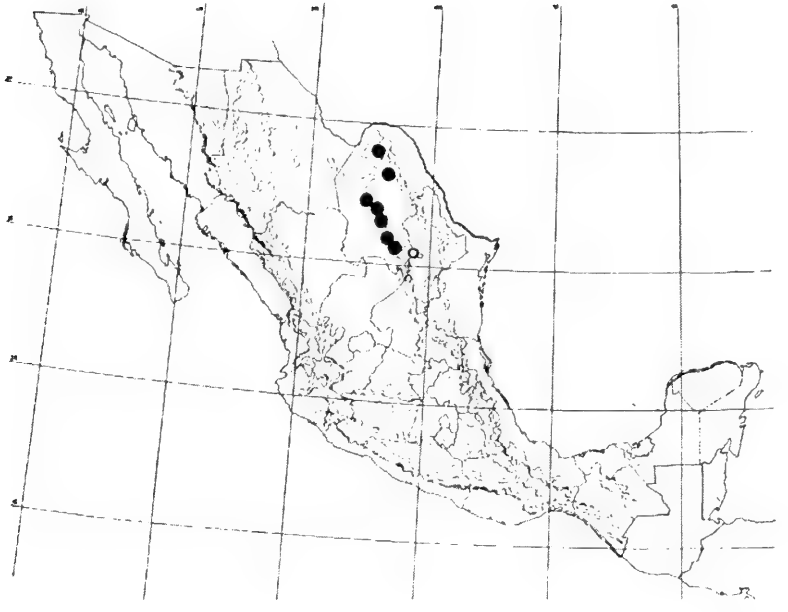

Fig. 2. Distribution of *Physaria mexicana* (dots), and *P. vigana* (circle).

STUDIES ON THE TAXONOMY, DISTRIBUTION, AND ABUNDANCE OF *THALICTRUM TEXANUM* (RANUNCULACEAE)

Jason R. Singhurst
Wildlife Diversity Program
Texas Parks and Wildlife Department
Austin, Texas 78704, U.S.A.
email Jason.Singhurst@tpwd.state.tx.us

David J. Rosen
S. M. Tracy Herbarium
Department of Rangeland Ecology & Management
Texas A&M University
College Station, Texas 77843-2126, U.S.A.

William R. Carr
The Nature Conservancy of Texas
San Antonio, Texas 78295-1440, U.S.A.

and

Walter C. Holmes
Department of Biology, Baylor University
Waco, Texas 76798-7388, U.S.A.

ABSTRACT

Field and herbarium studies show *Thalictrum texanum*, a species of conservation concern, to be distinct from both *T. arkansanum* and *T. debile*. The species, which is endemic to southeast Texas, is documented to occur in eight counties. A description of the species, list of exsiccatae, distribution map, discussion of its abundance and environment, and comments on its conservation status are also included.

KEY WORDS: Ranunculaceae, *Thalictrum*, Texas.

Currently, six species of the genus *Thalictrum* are recognized as occurring in Texas (Turner et al. 2003). Two of these, *T. dasycarpon* Fischer & Avé-Lallemant and *T. revolutum* DC., are of widespread distribution in southeastern United States, while *T. fendleri* Engelm. ex A. Gray is extensively distributed in southwestern United States and northern Mexico. *Thalictrum arkansanum* B. Boivin, a poorly known species, is limited in distribution, according to Park and Festerling (1997), to southern Arkansas, southeastern Oklahoma, and adjacent northeast Texas. However, the species is also reported to occur in Angelina, Houston, and San Augustine counties in central east Texas (Turner et al. 2003). The widespread eastern species, *T. thalictroides* (L.) A. J. Eames & B. Boivin, known from only Lamar and Red River counties in northeast Texas, has only recently been discovered in the state (Singhurst and Holmes 1998). Finally, the subject of this report, *Thalictrum texanum* (A. Gray) Small, is considered to be a Texas endemic (Correll and Johnston 1970) and, until now, was known only from three counties in the lower Brazos River area, northwest of the city of Houston (Turner et al. 2003). Park and Festerling (1997), who mention that the species is known from only two extant populations, consider the species to be of conservation concern. The species is said to be inconspicuous (Mahler 1983) and somewhat difficult to locate in the field (Park and Festerling 1997). Presently, *T. texanum* is classified as a plant species of concern (G2S2) (Poole et al. 2004), but there is concern that it may be the same as *T. debile* Buckley or better treated as a variety of that species. Park and Festerling (1997), under the treatment of *T. debile* Buckley, state that *T. texanum* is closely related to *T. arkansanum* and *T. debile* and that "The distinctions among the three species should be further studied."

The purpose of this paper is to (1) determine if there is sufficient reason to support the recognition of *Thalictrum texanum* as a species distinct from *T. arkansanum* and *T. debile*, and if so; (2) furnish information on its distribution and population; (3) discuss the general ecology of the species; and (4) make a recommendation as to the conservation status of the species.

METHODS

The study originated with examination of *Thalictrum* specimens in three herbaria (BAYLU, SBSC, and TEX/LL) to obtain a basic distribution of and the ecological preferences of the species. The holotype (*Hall 3*) was generously loaned to the researchers by the Gray Herbarium of Harvard University. These data were used to select prospective sites for field studies, which were concentrated in Austin, Brazoria, Brazos, Fayette, Grimes, Harris, Waller, and Washington counties. Actual field investigations were undertaken from 2002 to 2004. Although not directly part of the current study, previous field studies on *T. arkansanum*, which is also of conservation concern (Park and Festerling 1997), were conducted in 2000 by the senior author in Bowie, Delta, Lamar, and Red River counties in northeast Texas. The specimens collected proved to be invaluable to the study reported herewith.

Although not specifically an essential part of the present study, the authors felt that examination of the specimens from Angelina, Houston, and San Augustine counties in central east Texas referred to as *T. arkansanum* by Turner et al. 2003 would benefit the study. These specimens, examined at TAES and TAMU, are referable to *T. dasycarpon.*

The data and specimens collected were used to determine distribution, habitat characteristics, time of flowering, population estimates, range of morphological variation, and for comparisons with similar species.

TAXONOMY

THALICTRUM TEXANUM (A. Gray) Small, Fl. S.E. U.S. 446.1903

Type: **Texas**. Harris Co.: Moist prairies, Houston, 28 Mar 1872, *E. Hall 3* (Holotype: GH! Isotypes: NY, US).

Thalictrum debile Buckley var. *texanum* A. Gray in A. Gray et al., Syn. Fl. N. Amer. 1: 18. 1895.

Thalictrum debile Buckley var. *texana* A. Gray ex E. Hall, Plantae Texanae 3. 1873, *nomen nudum*.

Perennial, dioecious herbs. Roots fascicled, yellow when fresh, brown in age [black in age according to Correll and Johnston (1970)]. Stems ascending to erect, 14-35+ cm tall. Leaves generally clustered near the base, generally sparse above, reduced in size from base to apex, biternate, petioles 1-9 cm long, glabrate, irregularly angled, primary petiolules 0.5-4.0 cm long, secondary petiolules 1-10 mm long, those of the middle leaflet substantially longer than the lateral one, leaflets orbicular, subrotund, ovate, to reniform, 0.4-6.7(10) x 4-6.2(9) mm, margins entire or crenate to more often (especially the middle leaflet) shallowly to moderately cleft, bases cuneate, apices rounded and entire, surfaces glabrous, palmately 3 (5) nerved from the base, nerves prominent, exserted from the surface, lower surfaces generally whitened. Inflorescence a raceme, 2-10 cm long. Flowers: Sepals white to purplish, lanceolate to ovate, ♂ 1.7-3 mm , ♀ slightly smaller, stamens 10-14, filaments pinkish, 0.5-2.0 mm long, thin, anthers yellow, 1.4-1.8(2) mm long, apices pointed. Pistils ellipsoid, green, ca. 2.2 long, ribbed, styles/stigmas pinkish, ca. 0.8 mm long, curved, the stigmas linear, papillose, extending for most of the length of the style. Achenes 3-4 mm (or more) long, nearly sessile, ellipsoid-ovate, body 1.5–3.5 mm long, glabrous, slightly flattened, prominently 6–8 ribbed, beaks 0.5-1.0 mm long, straight, curved or reflexed, the upper half prominently papillose on one side. Seeds ellipsoid, flattened, glabrous, slightly smaller than the body of the achene.

Distribution. Clay-pan savannahs, alluvial plain terraces, and pimple mound prairies; lower Brazos River drainage (Austin, Brazoria, Brazos, Fayette, Grimes, Harris, Waller, and Washington counties);12–85 m (Fig. 1).

Phenology. Flowering (late January) February to May.

SPECIMENS EXAMINED: TEXAS. Austin Co.: Stephen F. Austin State Park, 80 m from SW corner of office building near entrance of park, 28 Feb 2003, *Singhurst 14613* (BAYLU); **Brazoria Co.:** Nash Ranch, W of Co. Rd. 25, about 8.7 mi. N of its intersection with TX Hwy 35 in West Columbia, 16 Mar 2004, *Rosen 2701* (BRIT, TEX); Nowatny Prairie, S of CR 18, about 0.4 mi. E of its intersection with Hwy 36, SE of the town of Damon, 21 Mar 2005, *Rosen 3286 and Singhurst* (SBSC); **Brazos Co.:** College Station, Texas, 22 Mar 1948, *Park s.n.* (TAMU); Frequent in moist woods along creek, 9.6 mi. SE of College Station, 26 Mar 1949, *Cory 55203* (SMU); 9 mi. S of A&M College, 11 Mar 1949, *Illige 119* (TAMU); Shady sandy soil, 8 mi. SE of College Station, 11 Mar 1949, *Whizenhunt 19* (TAMU); Damp sandy soil partially shaded area, 6 mi. S of College Station in roadside ditch along TX Hwy 6, 20 Mar 1957, *Cypert 106* (TAMU); Moist post oak woodland, 13 mi. S of College Station near TX Hwy 6, 15 Mar 1970, *Lonard and Bacon 2533* (SAT, SMU); Lick Creek Park SE of College Station, 17 Apr 1986, *Eaglesham 41* (TAMU); Between Lick Creek and Alum Creek, SW 1/4 of Lick Cr. Park, ca. 0.75-0.8 airmiles SSW of parking lot, 17 Mar 1992, *Carr and Manhart 11606* (TEX-LL); Lick Creek Park, W side of drainage ditch and E of Alum Creek ca. 500 ft. by air SW of its jct. with another major creek (either Lick Creek or a tributary), ca. 4.1-4.2 airmiles SSW of St. Rt. 30 bridge over Navasota River at Ferguson Crossing, 13 Apr 1999, *Carr and Linam 18084* (TEX-LL); **Fayette Co.:** Cummins Creek, 10 May 1849, *Wright s.n.* (GH); **Grimes Co.:** Cemetery of St. Holland Baptist Church, TX Hwy 6, 2 mi. N of FM 2, ca. 8 mi. S of Navasota, 23 Feb 1996, *Holmes 8035* (BAYLU); Ca. 500-800 ft. E of Co. Rd. 403 from a point ca. 3.7-3.8 road mi. N of its southern jct. with FM 3090, or ca. 4.1 airmiles N of jct. FM 3090 and St. Rt. 6 (on N side of Navasota), 25 Nov 1998, *Carr and Allen 17932* (TEX-LL); Ca. 500-800 ft. E of Co. Rd. 403 from a point ca. 3.7-3.8 road mi. of its southern jct. with F.M. 3090, or ca. 4.1 airmiles N of jct. FM 3090 and St. Rd. 6 (on N side of Navasota), 3 Mar 1999, *Carr 17939* (TEX-LL); Allen property, 16 Feb 2004, *Singhurst, Carr, Allen and Loring 12717* (BAYLU); Faqua tract, 16 Feb 2004, *Singhurst, Carr, Allen, and Loring 12718* (BAYLU); Roadside of Co. Rd. 403, 1.1 mi. NW of jct. of FM 3090, NE of Navasota, 8 Apr 2006, *Whitehead 66* (TAMU); **Harris Co.:** Moist prairies, Houston, 28 Mar 1872, *Hall s.n.* (GH); Tafton Academy school grounds and adjacent city park, 28 Feb 2003, *Johnson 1343*

(SBSC); Willow Park, jct. of Cliffwood and McDermed Roads, E at 10 m and 90 m under power-line, 28 Jan 2004, *Singhurst and Carr 12,540* (BAYLU); **Waller Co.:** Picnic area, N side of US Hwy 90, 0.4 mi. W of jct. of FM 1489, W of Brookshire, 10 Mar 1985, *Brown 8492* (ASTC, SMU); Small roadside park off of Hwy 90 W of Brookshire, 3 Mar 1986, Brown *9753* (SBSC); Picnic area on N side of US Hwy. 90, 0.4 mi. W of jct. FM 1489, just W of Brookshire, 26 Mar 1992, *Carr and Diamond 11657* (TEX-LL); N side of US Hwy 90, 0.4 mi. W of jct. FM 1489, just W of Brookshire, 21 Mar 1994, *Carr and Wolfe 13355* (TEX-LL); 2 mi. NW of Pattison, from intersection of Garrett Rd. and Buller Rd., head N 0.3 mi. to where Dry Branch crosses Buller Rd., 6 Apr 2003, *Johnson 1519* (SBSC); Ca. 0.3 mi. N of jct. of Garrett Rd. and Buller Rd., where Buller Rd. crosses Dry Branch, E side of Buller Rd., 28 Jan 2004, *Singhurst and Carr 12541* (BAYLU); **Washington Co.:** Washington Cemetery, Washington Cemetery Rd., 0.9-1.0 road mi. E of jct. TX Hwy 105, 17 Feb 2003, *Singhurst and Carr 11608* (BAYLU); S side of Washington Cemetery Rd. 0.9-1.0 road mi. E of its jct. with St. Rt. 105, just W of Washington on the Brazos State Park. 2.5-2.6 airmiles SSW of St. Rt. 105 bridge over Brazos River, *Carr and Singhurst 21545* (TEX-LL); Friden Church/Cemetery, 6 Feb 2004, *Holmes 12746 and Singhurst* (BAYLU).

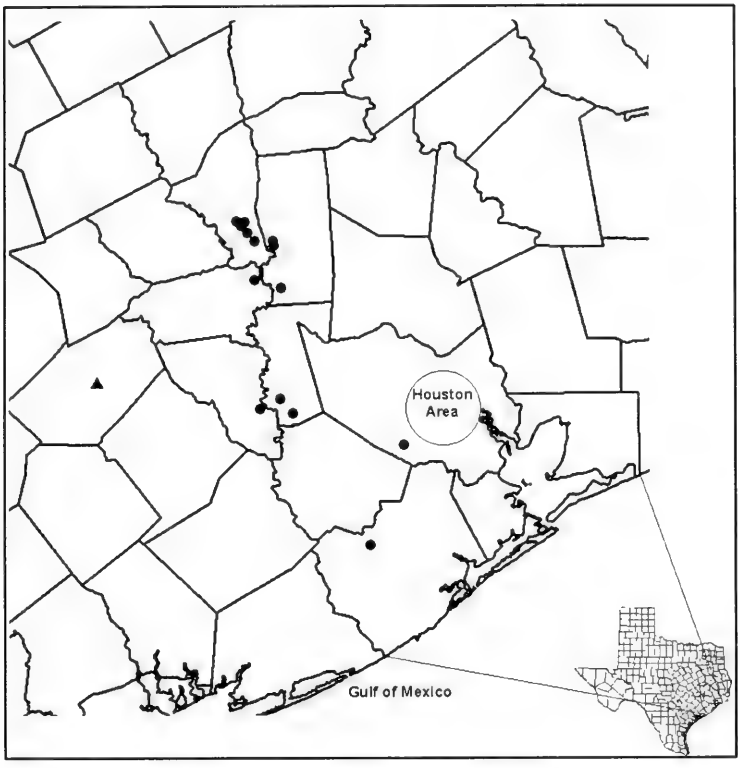

Figure 1. Distribution of *Thalictrum texanum* in Texas. Counties with dots (●) have extant populations. The record from Fayette County is historical, indicated by a triangle (▲).

DISCUSSION

Thalictrum texanum is regarded by Park and Festerling (1997) as being closely related to *T. debile* of Alabama, Georgia, and Mississippi, and to *T. arkansanum* of Arkansas, Oklahoma, and Texas. These species are characterized by tuberous roots, reclined to erect stems usually less than 45 cm tall, and leaflets of less than 15 mm in size. Apparently there is considerable difficulty in distinguishing the

three species, other than by distribution, which is further complicated by a lack of herbarium specimens, particularly of *T. texanum* and *T. arkansanum*. Park and Festerling (1997) mention that *T. texanum* is sometimes treated as a variety of *T. debile*, and that *T. arkansanum* possibly should be considered as a variety of *T. debile* also. The following characters from Park and Festerling's key may be used to distinguish the three species. *Thalictrum texanum* is characterized by its erect to ascending habit and ovoid achenes with beaks 0.5–1 mm long, while both *T. debile* and *T. arkansanum* have reclining to decumbent stems and oblong to elliptic-lanceolate achenes with beaks 1.3–2 mm long. Certainly the most impressive distinction involves leaflet characteristics. The terminal leaflet of *T. texanum* is smaller (length: $\bar{x}$ = 6.708, range 4–10, S.D. = 1.764013, n = 12; width: $\bar{x}$ = 6.225, range 5.2–9, S.D. = 1.19782, n = 12) and has whitened lower surfaces. *Thalictrum arkansanum* has larger leaflets (length: $\bar{x}$ = 12.09, range 8–18, S.D. = 2.954908, n = 27; width: $\bar{x}$ = 13.0, range 8–20, S.D. = 2.621954, n = 27) and generally lack the whitened lower surfaces. These characteristics, particularly the leaflet size, can be used to consistently distinguish *T. texanum* from *T. arkansanum*. The few specimens of *T. debile* examined had terminal leaflets that closely approximated those of *T. arkansanum,* suggesting uncertainty as to the distinction of *T. arkansanum*. This disposition agrees with the assessment discussed in Diggs et al. (1999). Thus, it is recommended that *T. texanum* be considered distinct from both *T. arkansanum* and *T. debile*.

The color of the roots is another characteristic that has been used to distinguish *Thalictrum texanum* from both *T. debile* and *T. arkansanum*. In Correll and Johnston (1970) in the key to species and in the description, the roots of *T. texanum* are described as becoming black upon drying, while those of *T. arkansanum* are described as brown. Essentially, Park and Festerling (1997) make the same statements in both the key to species and descriptions, but include *T. debile* ("roots brownish"), a species not within the territorial limits of Correll and Johnston (1970). Nonetheless, black roots have not been detected in any specimens of *Thalictrum texanum* consulted in this study. All roots were brown.

Until this report, *Thalictrum texanum* was known from historical locations and according to Park and Festerling (1997), only two extant populations. These were Lick Creek Park in Brazos County and an unnamed roadside park in Waller County. Turner et al. (2003) listed the species from Brazos, Grimes, and Waller counties. Historical sites included Harris County (the holotype) and an unknown location near Cummins Creek in Fayette County, based upon an 1849 collection made by Charles Wright. As a result of this present study, the species has been discovered in Austin, Brazoria, and Washington counties. Additionally, the species has been "relocated" in Harris County. To date, the species has been confirmed to occupy 13.4 ha (33 acres) in ten distinct populations in Austin, Brazos, Brazoria, Grimes, Harris, Waller, and Washington counties. General locations are given in the list of exsiccatae.

Thalictrum texanum is primarily distributed in the southeast portions of the Blackland Prairies and Post Oak Savannah vegetational areas at sites that may be described as transitional between the two areas; i.e., a blending of the characteristics of the two areas. One population (Brazoria County) is known from the Gulf Prairies and Marshes vegetation area. Generally, the species is found to occur on three distinct soil formations: clay-pan savannahs, alluvial plain terraces, and pimple mound prairies. More specifically, *T. texanum* occurs in woodlands and woodland margins on both uplands and creek terraces on soils with a surface layer of sandy loam over clay-pans.

At Stephen F. Austin State Park in Austin County, *Thalictrum texanum* inhabited an alluvial terrace dominated by *Carya illinoinensis*, *Ulmus crassifolia*, and *Plantanus occidentalis*. Non-woody vegetation included *Carex cherokeensis*, *Anemone heterophylla*, *Nothoscordum bivalve*, *Ranunculus carolinianus*, and *Scutellaria parvula*.

In Brazoria County, *Thalictrum texanum* was recently discovered on remnant prairies that are maintained by mowing and haying. The plants were growing on the fine sandy loam soils of pimple mounds. Pimple mounds were dominated by *Agrostis elliottiana*, *Agrostis hyemalis* var. *hyemalis*, *Carex meadii*, *Panicum* sp., and *Vulpia octoflora* var. *octoflora*. Other associates included *Anagallis minima*, *Anemone berlandieri*, *Dichondra carolinensis*, *Drosera*

brevifolia, *Erigeron tenuis*, *Euphorbia texana*, *Houstonia pusilla*, *Krigia dandelion*, *Lepuropetalon spathulatum*, *Nothoscordum bivalve*, *Oenothera laciniata*, *Polygala incarnata*, *Scleria ciliata* var. *glabra*, *Scutellaria parvula*, *Silphium gracile*, and *Triodanis perfoliata* var. *perfoliata*.

At Lick Creek Park in Brazos County, the species occurred along the margins of mostly deciduous woodland on an alluvial terrace, in partial shade of *Quercus nigra*, *Ulmus* sp., and *Ilex vomitoria*. Other vegetation included *Carex cherokeensis*, *Tridens flavus*, *Elephantopus carolinianus*, *Salvia lyrata*, *Verbesina virginica*, and *Schizachyrium scoparium*.

At the Allen Ranch in Grimes County, the species occurred in abundance along the margins of upland woodlands dominated by *Quercus stellata* and *Juniperus virginiana*. Shrub components included *Ilex vomitoria*, *Callicarpa americana* and *Vaccinium arboreum*. During the springtime, margins of the woodlands were dominated by *Carex complanata* and in the fall by *Schizachyrium scoparium*. The surface layer of the soil is slightly acidic fine sandy loam, while the upper part of the subsoil is slightly acid, nearly impermeable clay that ultimately produced a perched water table during the wet season (December to March). This combination keeps the soils very moist during periods of active growth of *Thalictrum texanum* and at the same time limits root penetration.

At the Washington Cemetery in Washington County, *Thalictrum texanum* occurred on a clay-pan savanna site dominated by mostly *Quercus stellata* subtended by small mottes of *Ilex vomitoria* and occasional clumps of *Schizachyrium scoparium*, *Saxifraga texana,* and *Claytonia virginica*.

SUMMARY

Based upon our findings, it is recommended that *Thalictrum texanum* be considered distinct from both *T. debile* and *T. arkansanum*, which may be conspecific. It is further suggested that *T. texanum* be considered to be of conservation concern, largely on the basis of the limited number of extant populations.

ACKNOWLEDGEMENTS

The authors are grateful to the herbaria whose specimens, in part, made this study possible and to Gail Allen, who granted access to her land in Grimes County. Both Dan Johnson and Larry Brown of SBSC very graciously provided information on the occurrence of *Thalictrum texanum* in Harris and Waller counties. We are also very thankful to the manuscript reviewers, Jackie Poole and David Riskind.

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NOTES ON THE *VERBESINA HINTONIORUM* (ASTERACEAE) COMPLEX OF NUEVO LEON, MEXICO

George S. Hinton
P.O. Box 603
Saltillo, Coahuila, Mexico 25000

B. L. Turner
Plant Resources Center
The University of Texas at Austin
Austin, Texas 78712
email billie@uts.cc.utexas.edu

ABSTRACT

In light of new collections, the *Verbesina hintoniorum* complex is reevaluated. The largely gypsophilic grouping is treated as composed of four species: *V. hintoniorum, V. aramberrana, V. zaragozana,* and *V. tamaunevana*; only the latter is nongypsophilic. A new taxon, ***V. zaragozana*** var. ***intermedia***, is described from gypseous outcrops north of Galeana, Nuevo Leon. A map showing the distribution of these taxa is provided.

KEY WORDS: *Verbesina*, Asteraceae, Mexico, Nuevo Leon

Turner (1998) published a new species, *Verbesina tamaunuevana,* belonging to the *V. hintoniorum* B.L. Turner complex. In this he noted that at least a few sheets of what he earlier identified as *V. zaragozana* appeared to be introgressed individuals or populations of *V. hintoniorum.* Additionally, he provided a preliminary map showing their distributions.

Upon reading his paper the senior author sent him an email message that read as follows:

> Attached is a copy of a map I made with my interpretation of the *Verbesina* species- which differs a bit from yours....I consider the soft tomentose [taxon]to be *V. zaragozana* and the stiffer hairy one

V. hintoniorum- if so, they are readily distinguishable in the field and I have yet to see any overlapping in their distribution. Unless the hairiness/tomentoseness is due to the variation in altitude- tomentose at lower altitudes and hairy at higher ones. With this in mind, the northernmost group is *V. zaragozana* in the municipality of Rayones, ranging from the gypsum near the village of Santa Rosa in the north to that around the villages of Rayones in the south. Next, *V. hintoniorum* ranges throughout the gypsum in the municipality of Galeana, starting around the the village of Galeana, down into San Jose del Rio and west to the hills below Cerro Potosi. Continuing south you again run into *V. zaragozana* on the gypsum in the municipalities of Aramberri and Zaragoza. The habitat of *V. aramberrana* is on gypsum to the west of of the sierra that runs south along the Y- Dr. Arroyo highway, except for one plant [that] I found growing in the *V. zaragozana* area near the town of Zaragozana, the only time I have seen an overlap in distribution for these species. *Verbesina tamaunuevana* is the southernmost square and the only member of the group that grows on non-gypsum...

The junior author was so impressed with his colleague's field knowledge of the taxa concerned that he reevaluated the *V. hintoniorum* complex, which is the purpose of the present communication.

Verbesina zaragozana var. **intermedia** G.B. Hinton & B.L. Turner, **var. nov.**

Verbesinae zaragozanae B.L. Turner var. *zaragozanae* similis sed differt capitulis aliquantum minoribus, flosculis radii brevioribus, et foliis plerumque angustioribus ac minus dense tomentosis.

Resembling *Verbesina zaragozana* B.L. Turner typica, but the heads somewhat smaller with shorter ray florets, and the leaves mostly narrower and less densely tomentose.

TYPE: **MEXICO. NUEVO LEON: Mpio. Galeana**, above El Nogal, "Stunted pine forest," 0.7 m high, 2250 m, 11 May 1983, *Hinton et al. 18093* (TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. NUEVO LEON**. **Mpio. Galeana**, Cienega del Toro to Santa Rosa, "Dominant species" on gypsum hillside, 1610 m, 3 Oct 1995, *Hinton et al. 25645* (TEX); 16.5 km N of Galeana along road to Rayones, 1100 m, 11 Nov. 1996, *Panero & Calzada 6876* (TEX). **Mpio Rayones,** 19 km from Galeana along road to Rayones, 1270 m, *Hinton et al. 20825* (TEX); along road from Galeana to Rayones, gypsum hillside, 1315 m, 24 Oct 1995, *Hinton et al. 25686* (TEX).

In the junior author's first account of *V. zaragozana* (Turner 1992) he noted that the species was partially sympatric with *V. hintoniorum* but did not appear to hybridize with it. His assertion that the two were sympatric was based upon material herein described as *V. z.* var. *intermedia.* Turner (1998), in his description of *V. tamanuevana*, noted "that at least a few collections of *V. hintoniorum*... have a vestiture" of *V. zaragozana*, but in other features resemble *V. hintoniorum,* hence the name var. *intermedia.* The variation concerned is perhaps derived from ancestral hybridization between *V. hintoniorum* and *V. zaragozana*, if not mere divergence from the latter.

ACKNOWLEDGEMENTS

Guy Nesom provided the Latin diagnosis and reviewed the manuscript, for which we are grateful.

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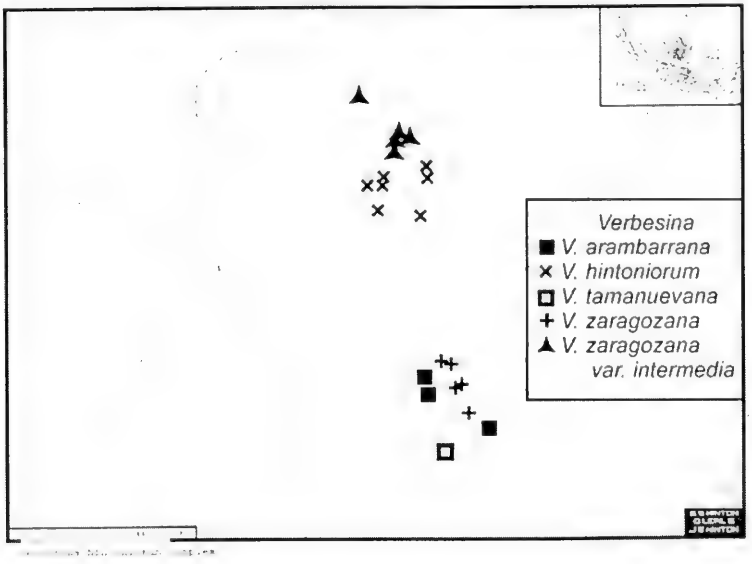

Fig. 1. Distribution of the *Verbesina hintoniorum* complex.

USING SEQUENCE INVERTED REPEATS (RAPDs) DATA IN SYSTEMAICS: POTENTIAL, PROBLEMS AND SOLUTIONS

Robert P. Adams
Baylor University, Gruver Lab, P.O. Box 727, Gruver, TX 79040 USA
email Robert_Adams@baylor.edu

ABSTRACT

Several cases are presented from *Juniperus* in which the use of RAPD (Random Amplified Polymorphic DNA = Sequence Inverted Repeats SIRs) have been very concordant with DNA sequence and biogeographic data. The use of the term 'Random' should be discouraged because the method is not 'Random' but depends on numerous inverted sequences found in DNA that accounts for hairpin loops important to define the tertiary structure of RNA and thence enzyme activity and specificity. SIRs (RAPDs) require very precise attention to laboratory procedures. Several suggestions are presented to aid in these procedures. Unlike DNA sequence data, SIRs (RAPDs) data should be analyzed by a multi-variate statistical method such as Principal Components or Principal Coordinates Analysis that can account for variation within taxa and treat this variation as error terms. Running replicates and/or sibs is critical to determine if lab supplies and equipment are operating at the peak efficiency.

KEY WORDS: Random Amplified Polymorphic DNA (RAPD), Sequence Inverted Repeats (SIRs), systematics, methods, *Juniperus*.

Obtaining reproducible RAPDs (Random Amplified Polymorphic DNA) or SIRs (Sequence Inverted Repeats) patterns can be very difficult. This has severely impacted the reputation of RAPD data. In fact, I recently had a manuscript rejected with only one comment "RAPDs are known to have problems with reproducibility. I find it inappropriate to base a key on these data" (actually the key used only morphological data, no RAPD data).

DNA fingerprinting methods (i.e., producing a bar-code of DNA bands) that utilize inverted repeats include RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and SSR (Simple Sequence Repeats, when using a single primer). Table

1 compares the basis, application and sequence knowledge needed for the

Table 1. Examples of DNA technology based methods for the analysis of genetic, breeding, and biodiversity studies [based in part from Henry, et al.(1997)].

Gene targeted	Primers	PCR bands	Application	Sequence data needed?
unknown	sequence inverted repeats (RAPDs) (SIRs)	several	bio-diversity, cultivar id., ssp/ var. id. mapping breeding	no, data mining from GenBank should lead to more general primers
various and inter-genic regions	inter-simple sequence repeats (ISSRs)	many	similar to RAPDs (above)	yes, based on known, sequence repeats but not for each taxon
unknown	M13F M13R (AFLPs)	many	similar to RAPDs (above)	no, but DNA must be cut with an enzyme and ligated to M13
unknown	consensus (intron/exon) (promoter/ exon)	many	similar to RAPDs (above)	yes, based on GenBank data mining, but not needed for each taxon

Table 1 (contd.)

Gene targeted	Primers bands	PCR	Application	Sequence data needed?
short simple sequence repeats [ex. $(GA)_{52}$] SSRs = STRs= microsatellites	simple repeats	one to a few	gene flow, parent id, hetero-zygosity estimates to find these	yes for the region bounding the SSR. Costly project
various genes	SNPs, single nucleotide polymorphisms	few	gene flow, parent id, biodiversity	yes, sequence needed for each sample
individual genes	based on sequence data from the taxon	one	species id, phylo-genetics	yes, sequence needed for each taxon

major kinds of DNA technology methods. One can see that those methods that don't require sequence knowledge are generally mostly utilized in gene mapping, populational studies, infraspecific variation, cultivar identification, etc. Studies concerning higher levels of relationships (between genera, families, etc.) almost exclusively utilize DNA sequencing.

It is important to examine the basis for the existence of sequence inverted repeats (SIRs) in DNA. Sequence inverted repeats (SIRs) in ssDNA form hairpin loops that are important for the control of gene transcription and subsequent protein processing (Brown, 2002). In addition, SIRs are extremely important in determining the tertiary structure of RNA. Figure 1 shows the structure of 16S rRNA (based on

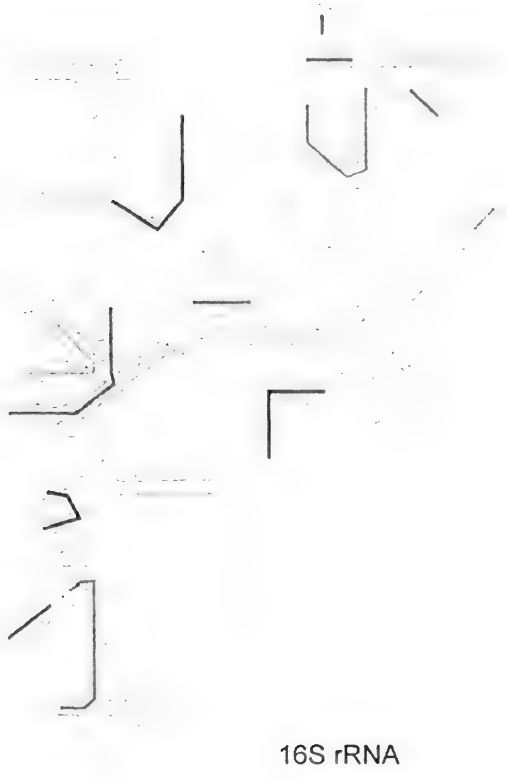

Fig. 1. Primary structure of 16s rRNA (from Noller, 2005). Note the prevalence of hairpin loops in the structure.

Noller, 2005). Hairpin loops are the dominant features of 16S rRNA primary structure. Interestingly, most of these hairpins are secured by inverted repeats of only 3 to 6 bp (Fig. 1). For 16S rRNA (Fig. 1), there appears to be only one 9 bp SIR. No SIR in 16S rRNA appears to be greater than 9 bp long. However, the frequency of hairpin structures in RNA is extensive (Noller, 2005), so SIRs of 10 bp and longer should be expected.

Figure 2 shows, diagrammatically, how sequence inverted repeats in DNA relate specifically to the formation of hairpin loops in RNA (Fig. 2). UBC 212 primer (shown in Fig. 2) is one of the 20 best

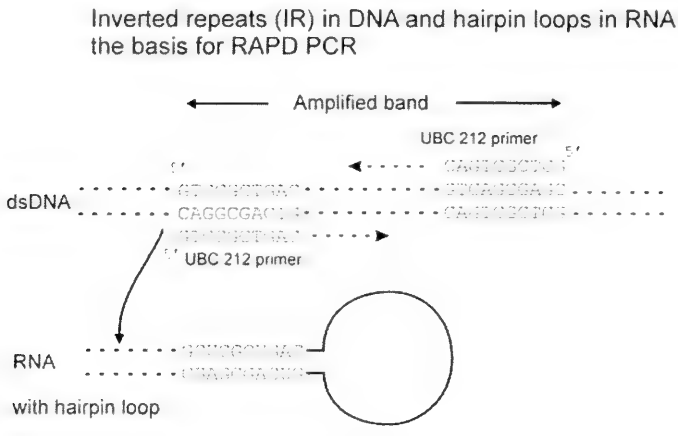

Fig. 2. Diagrammatic representation of an inverted repeat PCR and the relationship of the inverted repeat to a RNA hairpin loop.

primers found in our assays of 500 UBC primers screened on numerous plants, deer, fish and human DNAs (Table 2). PCR using the single UBC 212 primer results in an amplified band from this section of DNA (Fig. 2). The distance between the inverted repeats determines the size of the amplified band and also the hairpin loop size in the RNA (in this example). Of course, an additional priming site(s) may be present much farther downstream (even in an intron or in an inter-genic region) and would result in an additional, larger amplified band(s).

The use of single primers (inverted repeats) was co-discovered by Welsh and McClelland (1990) and Williams, et al. (1990). It is unfortunate that the terms 'random' and 'arbitrary' were used to describe the sequences of these primers, because we have discovered that the sequences are definitely neither 'random' nor 'arbitrary'. Beginning in 1990, our lab (Adams, Baylor University) began to screen 10 bp RAPD and 17-21 bp ISSR primers available in kits from the University of British Columbia (UBC). We have evaluated 500 RAPD and 100 ISSR primers for their ability to: 1. amplify DNA (from various sources, both

plants and animals); 2. obtain reproducible bands in replicate runs; 3. produce many bands; and 4. produce bands that are polymorphic between closely related species. These screenings revealed about 20 RAPD primers (4%) (Table 2) and 6 ISSR primers (6%) that met those criteria. It is now quite apparent that only certain sequences of IRs are common in genomes (about 4% of these tested).

Table 2. List of the most useful primers obtained from screening the UBC primer kits.

Name Sequence Best 20 primers:	Name Sequence Very variable (sensitive) primers:
116 TAC GAT GAC G	234 TCC ACG GAC G
134 AAC ACA CGA G	265 CAG CTG TTC A
153 GAG TCA CGA G	327 ATA CGG CGT C
184 CAA ACG GCA C	
204 TTC GGG CCG T	Other good primers
212 GCT GCG TGA C	131 GAA ACA GCG T
218 CTC AGC CCA G	237 CGA CCA GAG C
239 CTG AAG CGG A (conservative)	268 AGG CCG CTT A
244 CAG CGA ACC G	346 TAG GCG AAC G
249 GCA TCT ACC G	352 CAC AAC GGG T
250 CGA CAG TCC C	399 TTG CTG GGC G
338 CTC TGG CGG T	412 TGC GCC GGT G
347 TTG CTT GGC G	432 AGC GTC GAC T
375 CCG GAC ACG A	482 CTA TAG GCC G
376 CAG GAC ATC G	498 GAC AGT CCT G
389 CGC CCG CAG T	499 GGC CGA TGA T
391 GCG AAC CTC G	
413 GAG GCG GCG A	
431 CTG CGG GTC A	
478 CGA GCT GGT C	

Can these DNA bands be used in systematic studies? Figure 3 shows a comparison of two classifications of *Juniperus* species based on nrDNA (ITS) sequences and RAPD data. The correlation between these classifications was 0.95. Notice that whereas the ITS sequence data failed to resolve *J. macrocarpa*, *J. oxycedrus* and *J. o.* var. *badia*, these taxa were resolved in the RAPDs data (Fig. 3). Our experience in using several gene sequences for the phylogeny of *Juniperus* project (Schwarzbach et al., 2008), is that, in *Juniperus*, we still do not have gene sequences that can resolve very closely related species or many varieties.

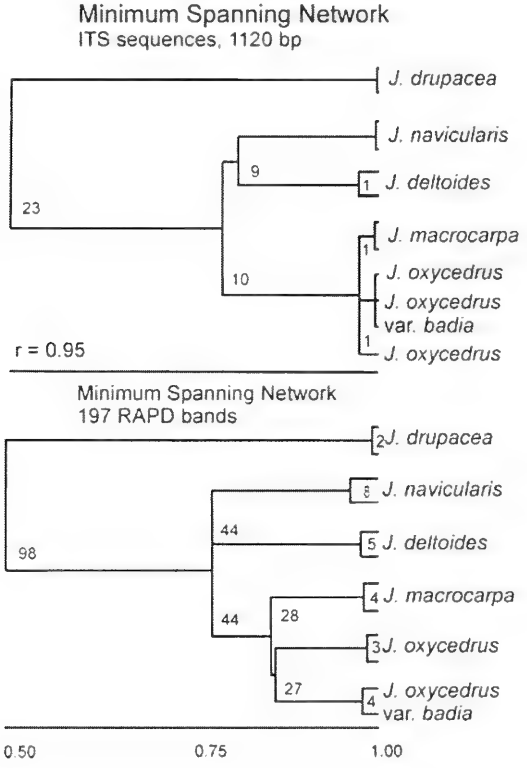

Fig. 3. Comparison of classifications based on ITS (nrDNA) sequences and RAPDs data (adapted from Adams et al., 2003). The correlation between the classifications is 0.95.

The juniper from the southwestern mountains of the Arabian peninsula has been called *J. excelsa* or *J. procera*. Principal coordinate analysis (PCO) using 121 RAPD bands to compute measures of similarity resulted in a very strong trend (Fig. 4, axis 1, 54%) that separated *J. excelsa* from the *J. procera* populations.

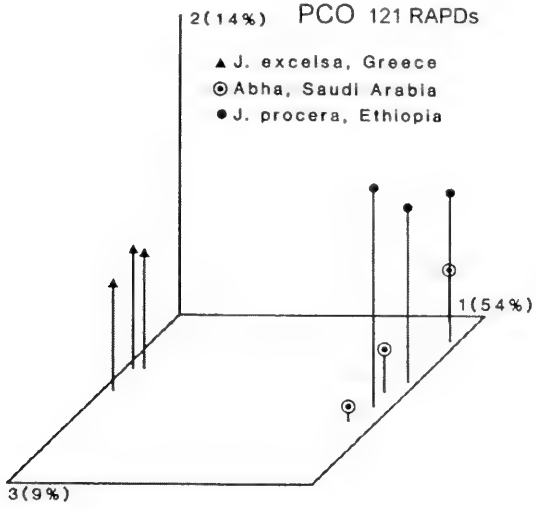

Fig. 4. PCO based on 121 RAPD bands for *J. excelsa*, Greece, putative *J. procera* from Abha, Saudi Arabia and *J. procera*, Ethiopia (adapted from Adams, et al., 1993).

Notice that 54% of the variation (axis 1) is due to the separation of *J. procera* from *J. excelsa* and that the putative *J. procera* plants from Abha all group with *J. procera* from Ethiopia.

Figure 5 shows a RAPD gel and sesquiterpenoids for *J. excelsa* (Greece), Abha, Saudi Arabia and *J. procera*, Ethiopia. It seems apparent that RAPDs can give the same kind of information as seen in the terpenoids (Fig. 5).

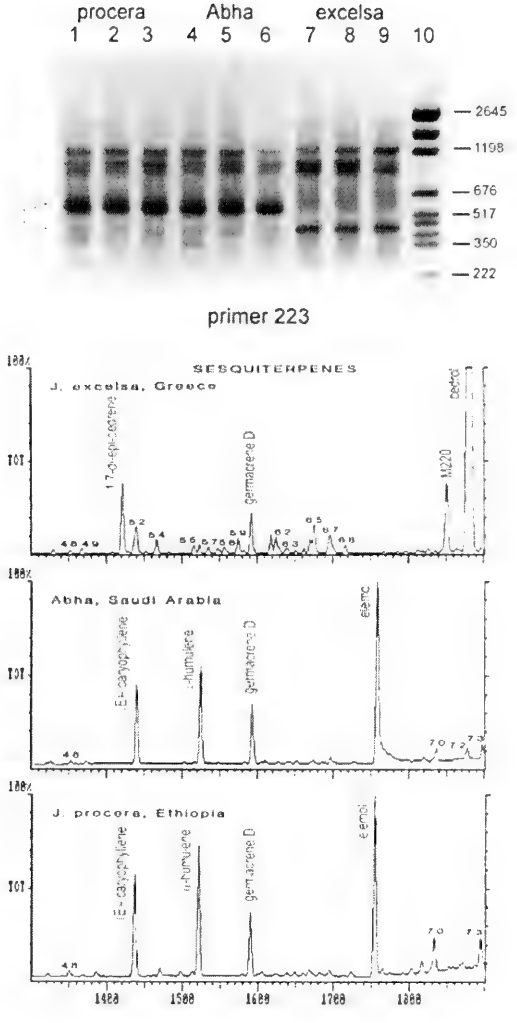

Fig. 5. Comparison of RAPD gel (primer 223) and sesquiterpenoids for taxa from Greece, Saudi Arabia, and Ethiopia (adapted from Adams, et al., 1993).

Figure 6 shows that RAPDs (Demeke, et al., 1992) analyzed by PCO perfectly reflect the famous U triangle (U, 1935) of relationships among *Brassica* species (based on chromosomal data).

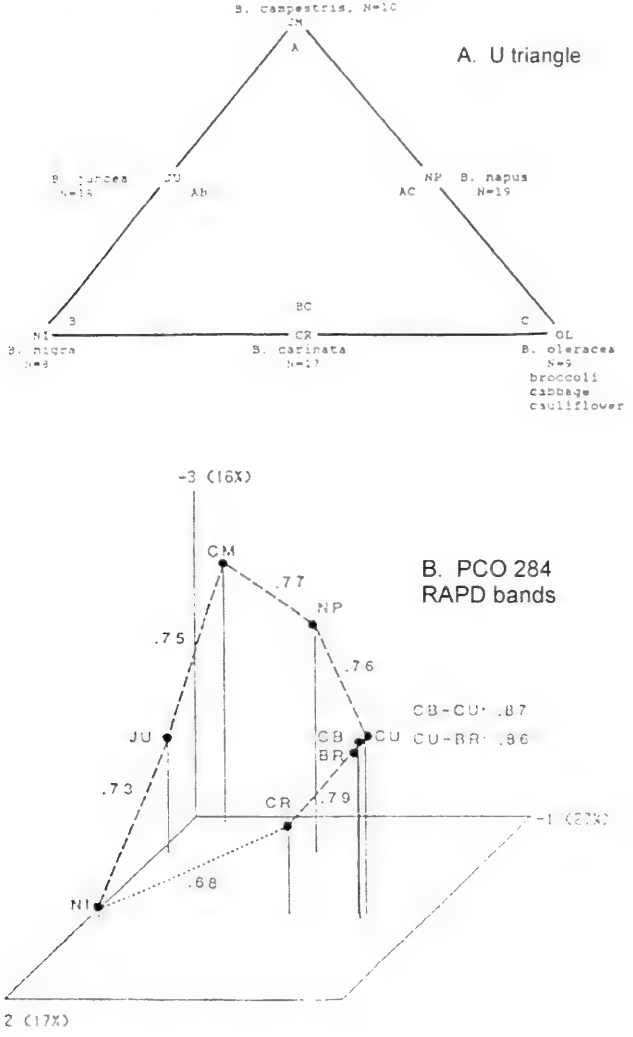

Fig. 6. Comparison of the U triangle and PCO based on RAPDs.

The U triangle of relationships among *Brassica* was based on chromosomal studies (U, 1935). Genotypes (with haplotypes) are represented by AA, AB, BB, BC, CC, and AC. B. PCO of the same *Brassica* taxa based on 284 RAPD bands. Notice the U triangle is perfectly represented by the RAPD data.

SOLUTIONS

There seem to be several factors that have enabled our lab to be able to utilize these kinds of data. First, we assume that there will be errors. There are some who think that data must be perfect in order to contain useful information. No data set (if very large) is likely to be free of errors (even DNA sequence data).

Second, we have developed laboratory methods that minimize errors. These methods are exhaustively discussed in Adams et al. (1998) and the reader is referred to www.juniperus.org to obtain a reprint. But it is worthwhile to consider a few of the major problems and solutions. The idea that RAPDs are not useful for systematics appears to have originated from a study by Penner et al. (1993) who investigated reproducibility in RAPDs using the same target DNA and primers in different laboratories. They found that the problems with reproducibility were mainly due to differences between PCR machines.

Another very influential study was that of Jones, et al. (1997) who sent *Populus* DNA, 2 primers, *Taq* polymerase (DynaZyme), and agarose to eight labs that used their own water, PCR tubes, and thermocyclers (3 of the most critical factors in RAPD PCR). They found only about a 75% reproducibility. Initially, in their AFLP tests, they had profiles that had 50% of the bands missing, but through practice, that improved to nearly perfect reproducibility (Jones et al., 1997). It seems odd that the reproducibility of RAPDs did not improve. However, it should be noted that there is no mention of water having been sent to each of the labs. The quality of water used in reactions is extremely critical. In addition, different thermocyclers were used and apparently not calibrated by external thermal meters.

We recently had to change suppliers for PCR tubes. Comparisons of tubes from 6 suppliers revealed that only 2 of the 6 manufacturers' PCR tubes gave the same results as our old 'discontinued' PCR tubes! In addition, we surveyed 4 sources of Taq and found tremendous variation in their products. So it seems that the Penner et al.

(1993) and Jones et al. (1997) studies did not adequately account for all variables that needed to be considered. In addition, variation among laboratory techniques alone likely accounted for considerable amounts of the variance among laboratories.

A very critical factor in PCR is the mixing of reagents. Pipetting is a source of many errors. Mixing reagents before and after pipetting is another critical step. To minimize errors due to pipetting very small amounts, Adams et al. (1998) investigated the stability of large amounts of RAPDs stock (ddwater, $MgCl_2$, 10x buffer, dNTPs and an entire vial of *Taq*). To maximize the potential for deterioration, the stock was stored at 22° C. They reported (Adams et al. 1998) no change in the RAPD amplification pattern after 4 days and only a slight reduction of band intensity after 60 days at 22° C! Of course, we store our RAPD stock at 4° C. The use of a large RAPD stock solution (enough to run all samples for one primer) and completely using an entire vial of *Taq* has greatly reduced variation (missing bands) in our replicated runs.

A second critical factor can be illustrated by my experience with a new post-doc from China. For several days he experienced failure to amplify for about 15 of 60 reactions. Finally, one day he obtained 60/60 perfect runs. Then the same success (60/60) for the next 5 days. I asked him if something was different. He replied, "I am now vortexing each PCR tube twice instead of once." Mixing of *Taq* and other reagents is extremely critical.

Because our lab has done considerable RAPD and sequencing, it is interesting to note that failure for a sequence to be generated is not unusual (or unexpected), but because the sequencing reaction did not give a credible sequence, one re-sequences the DNA without much thought. With sequencing, one can quickly see that the results are not credible and thus, the sample must be re-amplified and re-sequenced. But this is, intrinsically, more difficult to ascertain in PCR fingerprinting methods. Thus, it is important to have standard DNAs that generate standard profiles for each primer so these can be run as controls when new reagents are made, when a new thermocycler is used, etc. And it is critical to have multiple, genetically near-identical samples of each taxon to act as a reference to check that the amplification is credible. Obviously, if bands are not present or the larger bands are missing, one needs to re-run the RAPD analysis (we re-run in triplicate). It is almost impossible to overemphasize the attention

to detail that is required to do excellent RAPD analyses (see Adams, et al., 1998 for a very detailed discussion).

Thirdly, polysaccharides and other inhibitors (Pandey, et al. 1996; Adams et al., 1998) can cause problems in band amplifications. This is immediately apparent if the larger fragments are missing (2000-3500 bp). The DNA from *Juniperus flaccida*, extracted by the hot CTAB method, contains considerable inhibitors (Adams, et al., 1998) and could only be successfully amplified at a concentration of 250 pg of DNA (or less) per 15 µl PCR reaction. We now routinely use 300 pg of DNA per 15 µl PCR reaction. If the reaction does not amplify, we make serial dilutions of the DNA and run these until they amplify. In spite of better extraction kits, the easiest solution for inhibitor problems is to dilute the DNA.

Even if all these precautions are taken, there is still the problem of obtaining uniform, repeatable thermocycling conditions. We monitor every thermocycler with an external chart recorder and a temperature probe inside a control tube. This has enabled us to detect thermal cycling problems and correct them, and has also reduced our variation.

The lack of resolution of similar molecular weight bands on agarose (homology) is a problem (Rieseberg, 1996), but the use of similarity measures based on character differences, coupled with multivariate methods such as principal coordinates analysis (PCO), effectively eliminates this problem (see Adams and Rieseberg, 1998 for a detailed discussion).

In addition, it is important to recognize that not all bands generated are useful. There are some bands that, in replicated analyses, just tend to vary. By running several individuals from each taxon, collected in the same population, one can determine which bands are not representative of a population, variety or species. These can be eliminated. Our policy is, if in doubt, don't score the band. It is common that we discard 30 - 40% of the bands as being inconsistent, or just difficult to score. Demeke, et al. (1992) found in a study of *Brassica* that if fewer than 100 bands were utilized, the PCO ordination began to lose its correspondence to the U triangle (U, 1935). So it is necessary to start with 150 - 200 bands (generally using 15 - 18 primers that have been selected by intense screening, see Table 2).

Finally, it should be emphasized that multivariate statistical methods have the capability of accounting for error variance and are highly desirable for analysis. The movement in systematics to

parsimony tree building using sequence data has caused many to lose perspective that other kinds of data may require different methods for analyses. Certainly, those of us who have worked many years with secondary compound data are well aware of error variance and the need to factor data to remove (and account for) error variance. Perhaps a large part of the prejudice against the use of RAPD data for systematics is the result of a new generation of systematists who were not trained in the analysis of sampling errors.

It should be noted that not every person nor every lab can do this kind of analyses. I have had several students visit my lab that could just not do this kind of exacting work. I have had three students come from my colleague's lab in zoology, on separate occasions, for training in my lab. In each case, they obtained good results, but upon returning to their lab, they could never obtain reproducible results and abandoned the methods. Whether the problems were with their reagents, PCR tubes, water or thermocyclers was not determined.

Can RAPD data be used for systematics? From the examples above, I think it is impossible to explain the correlation between DNA sequence data and RAPD data classifications as chance. Laboratory procedures must be conducted at the highest standards using replicated analyses within each data set. Clearly, other kinds of data, including RAPDs, AFLP, ISSR, SSR, etc. can be utilized in systematics, but the methods of analyses need to be appropriate for the kinds of data concerned.

ACKNOWLEDGEMENTS

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NOMENCLATURAL CHANGES IN THE BRYACEAE (BRYOPSIDA) FOR NORTH AMERICA II.

John R. Spence
National Park Service, Glen Canyon National Recreation Area, P.O. Box 1507, Page, AZ 86040-1507, U.S.A. Email: John_Spence@nps.gov

ABSTRACT

An additional 25 new combinations in Bryaceae are made for the Bryophyte Flora of North America project in the genera *Gemmabryum*, *Imbribryum, Rosulabryum*, and *Ptychostomum.*

KEY WORDS: mosses, North America, Bryaceae, *Gemmabryum, Imbribryum, Ptychostomum, Rosulabryum.*

This paper represents the second installment of taxonomic and nomenclatural changes in the family Bryaceae for the Bryophyte Flora of North America. The first paper (Spence 2005) concentrated on the description of the new genera *Leptostomopsis* (C. Müll. Hal.) Spence & Ramsay and *Plagiobryoides* Spence as well as transfers to the re-instated genera *Haplodontium* Hampe and *Ptychostomum* Hornschuch. Below many additional species are transferred to the newly described genera *Gemmabryum* Spence & Ramsay and *Imbribryum* Pedersen and more transfers are made to *Ptychostomum* and *Rosulabryum* Spence.

Gemmabryum J.R. Spence & H.P. Ramsay

Gemmabryum was described by Spence and Ramsay (2005) for the Flora of Australia, with 25 species transferred. Although many of these also occur in North America, numerous additional species restricted to North America or the Northern Hemisphere exist as well that fit within the concept of *Gemmabryum*. An additional 10 species found in the Bryophyte Flora of North America region are here transferred to the genus.

Gemmabryum barnesii (J.B. Wood ex W.P. Schimper) J.R. Spence, **comb. nov.** Basionyn: *Bryum barnesii* J.B. Wood ex W.P. Shimper, Synopsis Muscorum Europaeorum, Ed. Secunda 471. 1876.

Gemmabryum bicolor (Dickson) J.R. Spence, **comb. nov.** Basionym: *Bryum bicolor* Dickson, Fasciculus Plantarum Cryptogamicarum Britanniae 4: 16. 1801.

Gemmabryum californicum (Sullivant) J.R. Spence, **comb. nov.** Basionym: *Bryum californicum* Sullivant, Exploration and Surveys for a Railroad Route from the Mississippi River to the Pacific Ocean, Description of the Mosses and Liverworts 4(5): 188. 6. 1856.

Gemmabryum demaretianum (Arts) J.R. Spence, **comb. nov.** Basionym: *Bryum demaretianum* Arts, Journal of Bryology 17: 263. 1992.

Gemmabryum gemmiferum (Wilczek & Demaret) J.R. Spence, **comb. nov.** Basionym: *Bryum gemmiferum* Wilczek & Demaret, Bulletin du Jardin Botanique National de Belgique 46: 529. f. 5. 1976.

Gemmabryum gemmilucens (Wilczek & Demaret) J.R. Spence, **comb. nov.** Basionym: *Bryum gemmilucens* Wilczek & Demaret, Bulletin du Jardin Botanique National de Belgique 46: 537. f. 9. 1976.

Gemmabryum mexicanum (Montagne) J.R. Spence, **comb. nov.** Basionym: *Brachymenium mexicanum* Montagne, Annales des Sciences Naturelles; Botanique, sér. 2, 9: 54. 1838.

Gemmabryum ruderale (Crundwell & Nyholm) J.R. Spence, **comb. nov.** Basionym: *Bryum ruderale* Crundwell & Nyholm, Botaniska Notiser 116: 95. 1963.

Gemmabryum valparaisense (Thériot) J.R. Spence, **comb. nov.**
Basionym: *Bryum valparaisense* Thériot, Revista Chilena de Historia Natural 21: 14. 4 f. 1. 1917.

Gemmabryum violaceum (Crundwell & Nyholm) J.R. Spence, **comb. nov.** Basionym: *Bryum violaceum* Crundwell & Nyholm, Botaniska Notiser 116: 94. 1963.

Imbribryum N. Pedersen
This genus was described based on molecular and morphological data by Pedersen and Hedenäs (2005) and Pedersen (2005). It consists primarily of the large imbricate-leaved species in *Bryum* section *Alpiniformia*. Four North American species are transferred.

Imbribryum gemmiparum (De Notaris) J.R. Spence, **comb. nov.**
Basionym: *Bryum gemmiparum* De Notaris, Commentario della Società Crittogamologica Italiana 2: 212 [112] [Cronaca Briol. Ital. 1: 25]. 1865.

Imbribryum microchaeton (Hampe) J.R. Spence, **comb. nov.**
Basionym: *Bryum microchaeton* Hampe, Annales des Sciences Naturelles; Botanique, sér. 5, 4: 342. 1865.

Imbribryum mildeanum (Juratzka) J.R. Spence, **comb. nov.** Basionym: *Bryum mildeanum* Juratzka, Verhandlungen der Zoologisch-botanischen Gesellschaft in Wein 12: 967. 1862.

Imbribryum miniatum (Lesquereux) J.R. Spence, **comb. nov.**
Basionym: *Bryum miniatum* Lesquereux, Memoirs of the California Academy of Sciences 1: 23. 1868.

Ptychostomum **Hornschuch**
Continuing fieldwork and examination of collections has documented or confirmed the existence of several additional species in North America. The following 10 species found in North America are transferred to the genus.

Ptychostomum acutiforme (Limpricht) J.R. Spence **comb. nov**. Basionym: *Bryum acutiforme* Limpricht, Tromsø Museums Aarshefter 21-22: 156. 1901.

Ptychostomum axel-blyttii (H. Philibert) J.R. Spence, **comb. nov**. Basionym: *Bryum axel-blyttii* H. Philibert, Revue Bryologique 16: 61. 1889.

Ptychostomum archangelicum (Bruch & W.P. Schimper in B.S.G.) J.R. Spence, **comb. nov**. Basionym: *Bryum archangelicum* Bruch & Schimper in B.S.G., Bryol. Eur. 4: 153, pl. 333. 1846.

Ptychostomum badium (Bridel) J.R. Spence, **comb. nov**. Basionym: *Bryum caespiticium* var. *badium* Bridel, Bryol. Univ. 1: 850. 1827.

Ptychostomum bryoides (R. Brown) J.R. Spence **comb. nov**. Basionym: *Pohlia bryoides* R. Brown, Chloris Melvilliana 38: 1823.

Ptychostomum funkii (Schwägrichen) J.R. Spence **comb. nov**. Basionym: *Bryum funkii* Schwägrichen, Species Muscorum Frondosorum, Supplementum Primum 2: 89. pl. 69. 1816.

Ptychostomum kunzei (Hornschuch) J.R. Spence **comb. nov**. Basionym: *Bryum kunzei* Hornschuch, Flora 2(1): 90. 1819.

Ptychostomum nitidulum (Lindberg) J.R. Spence **comb. nov**. Basionym: *Bryum nitidulum* Lindberg, Öfversigt of Förhandlinger: Kongl. Svenska Vetenskaps-Akademien 23: 545. 1866.

Ptychostomum ovatum (Hedwig) J.R. Spence, **comb. nov.** Basionym: *Gymnostomum ovatum* Hedwig, Species Muscorum Frondsorum 31. pl. 2: 1-3. 1801.

Ptychostomum teres (Lindberg) J.R. Spence **comb. nov**. Basionym: *Bryum teres* Lindberg, Öfversigt af Förhandlingar: Kongl. Svenska Vetenskaps-Akademien 23: 545. 1866.

Rosulabryum J.R. Spence

One species is transferred to this genus as there is a need for the name to be made available quickly for other pending studies, including the forthcoming Moss Flora of Colorado. The status and taxonomy of the genus in North America will be analyzed in a separate paper (Spence in prep.).

Rosulabryum flaccidum (Bridel) J.R. Spence, **comb. nov**. Basionym: *Bryum flaccidum* Bridel, Bryologia Universa 1: 667. 1826.

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NEW COMBINATIONS IN *DIMEROSTEMMA* (ASTERACEAE: HELIANTHEAE – ECLIPTINAE)

Marta Dias de Moraes
Centro de Ciências Biológicas e da Natureza
Universidade Federal do Acre, Campus Floresta, Rua Paraná 4016,
Cruzeiro do Sul, CEP 69980-000, Ac, Brazil.
mdmoraes@yahoo.com.br.

José L. Panero
Section of Integrative Biology, The University of Texas, 1 University Station, A6700, Austin, TX 78712, USA.

João Semir
Departamento de Botânica, Instituto de Biologia, UNICAMP, Caixa Postal 6109, Campinas, CEP 13083-970, SP, Brazil.

ABSTRACT

The use of ray flower sexuality in subtribe Ecliptinae has been a convenient character to split large or taxonomically challenging genera into smaller units. This approach has resulted in the placement of sister species in different genera. The genera *Dimerostemma* and *Angelphytum* from South America are examples, species having pistillate ray flowers placed in *Angelphytum* G. M. Barroso and those with sterile ray flowers in *Dimerostemma* Cass. A molecular phylogenetic study has shown that *Dimerostemma* is paraphyletic with the exclusion of *Angelphytum* and that the two genera comprise a strongly supported monophyletic group. This result and the lack of enough morphological evidence for the division between the two genera lead us to consider *Angelphytum* a synonym of *Dimerostemma*. All species of *Angelphytum* are formally transferred herein to *Dimerostemma*, summing up 17 new combinations. The genus *Dimerostemma* is easily separated from other similar composites mainly by cypselae, the pappus consisting of stout, triquetrous, tapering awns (sometimes lacking) fused to an unconstricted crown.

KEY WORDS: Asteraceae, Heliantheae, Ecliptinae, *Dimerostemma*.

The genera *Dimerostemma* Cass. and *Angelphytum* G. M. Barroso have collectively 29 species endemic to South America, with the highest concentration of species found in central-western Brazil. The genera belong to subtribe Ecliptinae of tribe Heliantheae, a group of 49 genera and approximately 380 species of shrubs and trees distributed mostly in the Neotropical region (Panero, 2007). *Dimerostemma*, originally monotypic, was emended by Blake (1917) to include species from *Oyedaea* DC. that lacked squamellae in their pappi. Robinson (1984a), while revising the generic limits of *Oyedaea*, placed in *Dimerostemma* all the Brazilian species, leaving within *Oyedaea* most species restricted to the Andes that bear a distinct neck at the apex of the cypsela.

In the original description of *Angelphytum*, Barroso (1980) considered the eradiate heads with peripheral fertile florets to differentiate her new genus from both *Zexmenia* La Lave & Lex. (with radiate heads) and *Dimerostemma* (bearing peripheral sterile florets). Robinson (1984b) transferred to *Angelphytum* all the Brazilian species with radiate heads that had been previously placed in *Zexmenia*. He justified his decision by alluding to the unreliability of eradiate heads as a distinguishing character, given that it occurs elsewhere in Ecliptinae, as in *Zexmenia* and *Wedelia* Jacq. (including *Aspilia* Thouars). With these new combinations and the inclusion of new species described since these taxonomic studies, the taxonomic limits between *Angelphytum* and *Dimerostemma* have become difficult to ascertain. The only morphological difference between these two genera is the peripheral or ray flower sexuality, sterile in *Dimerostemma*, fertile in *Angelphytum*. Except for this character, *Angelphytum* is essentially identical to *Dimerostemma*.

To elucidate the relationships of *Dimerostemma* and *Angelphytum*, a phylogenetic study based on ITS and ETS sequence data for the majority of the members of the Ecliptinae was constructed by Moraes (2004). In this study, the nine species of *Dimerostemma* and nine species of *Angelphytum* sampled are collectively revealed as a strongly supported monophyletic group. The only annual species of the group, *Dimerostemma annuum*, is basal to two main subclades containing each a combination of species of both genera. The generic type, *Angelphytum matogrossense* G. M. Barroso is clustered with most species of *Dimerostemma*. The results from molecular studies and the lack of any obvious morphological feature that can be used as a

synapomorphy to separate the main clades of the group, have led us to propose the placement of *Angelphytum* within *Dimerostemma.*

Dimerostemma is characterized by an involucre with an outer series of leaf-like phyllaries, disc corollas with cylindric upper throats, and by inner cypselae obovate in outline, cuneate toward base, usually laterally flattened, and mostly winged on the margins. The pappus is the most reliable feature for distinguishing *Dimerostemma* among ecliptinous genera. The pappus of *Dimerostemma* is coroniform with awns mostly well developed that are distinct in being stout, triquetrous, tapering and continuous with the margins of the cypsela. *Dimerostemma* is the only member in the subtribe that is differentiated by the extension of phytomelanin from the body of the cypsela to the base of the awns. The crown is inserted directly on the apex of the cypsela body, not raised on a rostrum as in *Oyedaea* , *Zexmenia*, and *Wedelia.*

To formalize the transfer of *Angelphytum* into *Dimerostemma*, the following new combinations are required:

Dimerostemma apense (Chodat) M. D. Moraes, **comb. nov.**
Basionym: *Aspilia apense* Chodat, Bull. Herb. Boissier sér. 2 (3): 721. 1903.

Dimerostemma arnottii (Baker) M. D. Moraes, **comb. nov**.
Basionym: *Verbesina arnottii* Baker in Martius, Fl. bras. 6 (3): 215. 1884.

Dimerostemma aspilioides (Griseb.) M. D. Moraes, **comb. nov.**
Basionym: *Verbesina aspilioides* Griseb., Abh. Königl. Ges. Wiss. Göttingen 24: 194. 1879.

Dimerostemma bahiense (H. Rob.) M. D. Moraes, **comb. nov.**
Basionym: *Angelphytum bahiense* H. Rob., Proc. Biol. Soc. Wash. 97 (4): 966. 1984.

Dimerostemma goyazense (Gardner) M. D. Moraes, **comb. nov.**
Basionym: *Lipochaeta goyazensis* Gardner, Lond. J. Bot. 7: 406. 1948.

Dimerostemma grisebachii (Baker) M. D. Moraes, **comb. nov.** Basionym: *Verbesina grisebachii* Baker in Martius, Fl. bras. 6.(3): 214. 1884.

Dimerostemma hatschbachii (H. Rob.) M. D. Moraes, **comb. nov.** Basionym: *Angelphytum hatschbachii* H. Rob., Proc. Biol. Soc. Wash. 97 (4): 967. 1984.

Dimerostemma herzogii (Hassl.) M. D. Moraes, **comb. nov.** Basionym: *Zexmenia herzogii* Hassl., Repert. Spec. Nov. Regni Veg. 7: 357. 1909.

Dimerostemma hieronymi (Hassl.) M. D. Moraes, **comb. nov.** Basionym: *Zexmenia hieronymi* Hassl., Repert. Spec. Nov. Regni Veg. 14: 157. 1915.

Dimerostemma indutum (Chodat) M. D. Moraes, **comb. nov.** Basionym: *Aspilia induta* Chodat, Bull. Herb. Boissier sér. 2 (3): 720. 1903.

Dimerostemma matogrossense (G. M. Barroso) M. D. Moraes, **comb. nov.** Basionym: *Angelphytum matogrossense* G. M. Barroso, Bol. Soc. Argent. Bot. 19 (1-2) 9. 1980.

Dimerostemma myrtifolium (Chodat) M. D. Moraes, **comb. nov.** Basionym: *Verbesina myrtifolia* Chodat, Bull. Herb. Boissier sér. 2 (2): 393. 1902.

Dimerostemma oppositifolium (A. A. Sáenz) M. D. Moraes, **comb. nov.** Basionym: *Zexmenia oppositifolia* A. A. Sáenz, Hickenia 1(54): 285. 1982.

Dimerostemma paraguariense (Chodat) M. D. Moraes, **comb. nov.** Basionym: *Verbesina paraguariensis* Chodat, Bull. Herb. Boissier sér. 2 (3): 722. 1984.

Dimerostemma pseudosilphoides (Hassl.) M. D. Moraes, **comb. nov.** Basionym: *Zexmenia pseudosilphioides* Hassl., Repert. Spec. Nov. Regni Veg. 14: 263. 1916.

Dimerostemma reitzii (H. Rob.) M. D. Moraes, **comb. nov.**
Basionym: *Angelphytum reitzii* H. Rob., Proc. Biol. Soc. Wash. 97 (4): 968. 1984.

Dimerostemma tenuifolium (Hassl.) M. D. Moraes, **comb. nov.**
Basionym: *Zexmenia tenuifolia* Hassl., Repert. Spec. Nov. Regni Veg. 14: 178. 1915.

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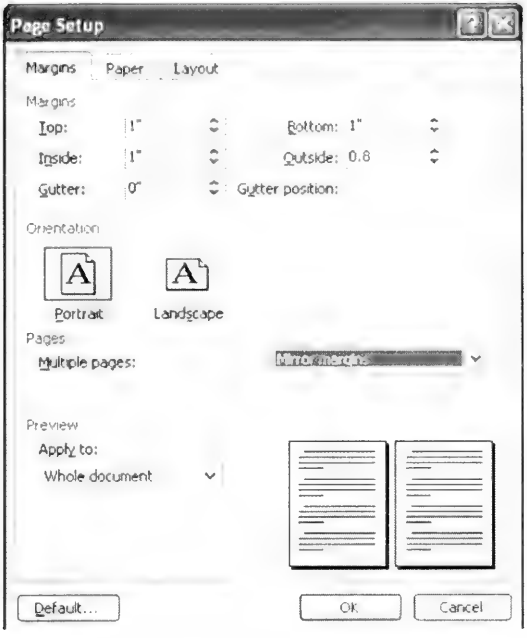

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Robert P. Adams
Biology Department, Baylor University, Waco, TX 79040, USA
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ACKNOWLEDGEMENTS

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LITERATURE CITED

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Journal article:

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 1977. Chemosystematics - Analyses of populational differentiation and variability of ancestral and recent populations of *Juniperus ashei*. Ann. Mo. Bot. Gard. 64: 184-209.

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